



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C12N 15/12, C07K 14/47	A2	(11) International Publication Number: WO 99/06554 (43) International Publication Date: 11 February 1999 (11.02.99)
(21) International Application Number: PCT/IB98/01238 (22) International Filing Date: 31 July 1998 (31.07.98) (30) Priority Data: 08/905,134 1 August 1997 (01.08.97) US (71) Applicant (for all designated States except US): GENSET [FR/FR]; 24, rue Royale, F-75008 Paris (FR). (72) Inventors; and (75) Inventors/Applicants (for US only): DUMAS MILNE EDWARDS, Jean-Baptiste [FR/FR]; 8, rue Grégoire de Tours, F-75006 Paris (FR). DUCLERT, Aymeric [FR/FR]; 6 ter, rue Victorine, F-94100 Saint-Maur (FR). LACROIX, Bruno [FR/FR]; 93, route de Vourles, F-69230 Saint-Genis Laval (FR). (74) Agents: MARTIN, Jean-Jacques et al.; Cabinet Regimbeau, 26, avenue Kléber, F-75116 Paris (FR).		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>Without international search report and to be republished upon receipt of that report.</i>
(54) Title: 5' ESTs FOR SECRETED PROTEINS EXPRESSED IN MUSCLE AND OTHER MESODERMAL TISSUES (57) Abstract The sequences of 5' ESTs derived from mRNAs encoding secreted proteins are disclosed. The 5' ESTs may be to obtain cDNAs and genomic DNAs corresponding to the 5' ESTs. The 5' ESTs may also be used in diagnostic, forensic, gene therapy, and chromosome mapping procedures. Upstream regulatory sequences may also be obtained using the 5' ESTs. The 5' ESTs may also be used to design expression vectors and secretion vectors.		

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P/ INT COOPERATION TREA

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

From the INTERNATIONAL BUREAU

To:

Assistant Commissioner for Patents
United States Patent and Trademark
Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 11 September 2000 (11.09.00)	
International application No. PCT/US99/30408	Applicant's or agent's file reference PF-0637 PCT
International filing date (day/month/year) 10 December 1999 (10.12.99)	Priority date (day/month/year) 11 December 1998 (11.12.98)
Applicant TANG, Y., Tom et al	

1. The designated Office is hereby notified of its election made:

☒ in the demand filed with the International Preliminary Examining Authority on:
26 June 2000 (26.06.00)

☐ in a notice effecting later election filed with the International Bureau on:

2. The election ☒ was
☐ was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No.: (41-22) 740.14.35	Authorized officer Nestor Santesso Telephone No.: (41-22) 338.83.38
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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁷ : C12N 15/12, C07K 14/47, C12Q 1/68, A61K 38/17, C07K 16/18		A2	(11) International Publication Number: WO 00/34477
			(43) International Publication Date: 15 June 2000 (15.06.00)
(21) International Application Number: PCT/US99/30408 (22) International Filing Date: 10 December 1999 (10.12.99) (30) Priority Data: Not furnished 11 December 1998 (11.12.98) US 09/210,083 11 December 1998 (11.12.98) US 60/119,365 9 February 1999 (09.02.99) US 60/124,687 16 March 1999 (16.03.99) US (63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Applications US Not furnished (CIP) Filed on 11 December 1998 (11.12.98) US 60/119,365 (CIP) Filed on 9 February 1999 (09.02.99) US 60/124,687 (CIP) Filed on 16 March 1999 (16.03.99) US 09/210,083 (CIP) Filed on 11 December 1998 (11.12.98) (71) Applicant (for all designated States except US): INCYTE PHARMACEUTICALS, INC. [US/US]; 3174 Porter Drive, Palo Alto, CA 94304 (US).		(72) Inventors; and (75) Inventors/Applicants (for US only): TANG, Y., Tom [CN/US]; 4230 Ranwick Court, San Jose, CA 95118 (US). YUE, Henry [US/US]; 826 Lois Avenue, Sunnyvale, CA 94087 (US). BAUGHN, Mariah, R. [US/US]; 14244 Santiago Road, San Leandro, CA 94577 (US). HILLMAN, Jennifer, L. [US/US]; 230 Monroe Drive #12, Mountain View, CA 94040 (US). LAL, Preeti [IN/US]; 2382 Lass Drive, Santa Clara, CA 95054 (US). AU-YOUNG, Janice [US/US]; 233 Golden Eagle Lane, Brisbane, CA 94005 (US). YANG, Junming [CN/US]; 7136 Clarendon Street, San Jose, CA 95129 (US). LU, Dyung, Aina, M. [US/US]; 55 Park Belmont Place, San Jose, CA 95136 (US). AZIMZAI, Yalda [US/US]; 2045 Rock Springs Drive, Hayward, CA 94545 (US). (74) Agents: BILLINGS, Lucy, J. et al.; Incyte Pharmaceuticals, Inc., 3174 Porter Drive, Palo Alto, CA 94304 (US). (81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published Without international search report and to be republished upon receipt of that report.	

(54) Title: **NEURON-ASSOCIATED PROTEINS**

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1  MA-----GSPSRAAGRRLLQLP----- 2417014
1  MEFSLLLPRL E CNGAISAHRENLRLLPGSSDS GI 3002527

17 -----L L C L F L O----- 2417014
31 PASASPVAGITGMCTHARLI LYFELVEMEF GI 3002527

24 ---GATAVLFAVF-----VRYNHKT 2417014
61 LHVGQAGLELPTSDDPSVSASQSA RYRTGH GI 3002527

41 DAAL-----WH----- 2417014
91 HARLCLANFCGRNRVSLMCPSWSP ELKQST GI 3002527

47 -----RSNHSNADNEFYFRY---PKESHHS 2417014
121 CLSLPKCWDYRRAAVPGLFILFFLRHRCPT GI 3002527

68 VAQAGVQRRNLGSLQPSPPR----- 2417014
151 LTQDEVQWCDHSLSLQPS TPEIKHP PASASQ GI 3002527

88 -----W-----SFALVA 2417014
181 VAGTKDMHHYT W L I F I F I F N F L R Q S L N S V T GI 3002527

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(57) Abstract

The invention provides human neuron-associated proteins (NEUAP) and polynucleotides which identify and encode NEUAP. The invention also provides expression vectors, host cells, antibodies, agonists, and antagonists. The invention also provides methods for diagnosing, treating, or preventing disorders associated with expression of NEUAP.

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

8

Applicant's or agent's file reference PF-0637 PCT	FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. PCT/US99/30408	International filing date (day/month/year) 10 DECEMBER 1999	Priority date (day/month/year) 11 DECEMBER 1998
International Patent Classification (IPC) or national classification and IPC Please See Supplemental Sheet.		
Applicant INCYTE PHARMACEUTICALS, INC.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 5 sheets.
- ☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority. (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).
- These annexes consist of a total of 0 sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☒ Non-establishment of report with regard to novelty, inventive step or industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability, citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☐ Certain observations on the international application

Date of submission of the demand 26 JUNE 2000	Date of completion of this report 07 MARCH 2002
Name and mailing address of the IPEA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer <i>Sharon L. Turner</i> SHARON L. TURNER
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

I. Basis of the report1. With regard to the **elements** of the international application:*

- ☒ the international application as originally filed
- ☒ the description:
pages 1-83 , as originally filed
pages NONE , filed with the demand
pages NONE , filed with the letter of _____
- ☒ the claims:
pages 84-85 , as originally filed
pages NONE , as amended (together with any statement) under Article 19
pages NONE , filed with the demand
pages NONE , filed with the letter of _____
- ☒ the drawings:
pages 1-5 , as originally filed
pages NONE , filed with the demand
pages NONE , filed with the letter of _____
- ☒ the sequence listing part of the description:
pages 1-53 , as originally filed
pages NONE , filed with the demand
pages NONE , filed with the letter of _____

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

- ☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☒ contained in the international application in printed form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. ☒ The amendments have resulted in the cancellation of:

- ☒ the description, pages NONE
- ☒ the claims, Nos. NONE
- ☒ the drawings, sheets/fig NONE

5. ☐ This report has been drawn as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).

**Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been and will not be examined in respect of:

- ☐ the entire international application.
- ☒ claims Nos. 17-20 and sequences as indicated in search report

because:

- ☐ the said international application, or the said claim Nos. _ relate to the following subject matter which does not require international preliminary examination (*specify*).

- ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. _ are so unclear that no meaningful opinion could be formed (*specify*).

- ☐ the claims, or said claims Nos. _ are so inadequately supported by the description that no meaningful opinion could be formed.

- ☒ no international search report has been established for said claims Nos. 17-20.

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

- ☐ the written form has not been furnished or does not comply with the standard.
- ☐ the computer readable form has not been furnished or does not comply with the standard.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.

PCT/US99/30408

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. statement

Novelty (N)	Claims	<u>7-8, 12-16</u>	YES
	Claims	<u>1-6, 9-11</u>	NO
Inventive Step (IS)	Claims	<u>7-8, 12-16</u>	YES
	Claims	<u>1-6, 9-11</u>	NO
Industrial Applicability (IA)	Claims	<u>1-16</u>	YES
	Claims	<u>NONE</u>	NO

2. citations and explanations (Rule 70.7)

Claims 1-6, 9 and 10 lack novelty under PCT Article 33(2) as being anticipated by LaCroix et al., WO 99/06554 as the reference teaches SEQ ID NOs 45 and 313 which share identity as indicated in the International Search Report. In the absence of computer readable format for the sequences in the PCT and parent documents applicants priority cannot be established and the reference is cited accordingly.

Claims 3-6 and 9-11 lack novelty under PCT Article 33(2) as being anticipated by NCI CGAP EMEST Database Accession A1022447, 19 June 1998 which shares identity as indicated in the International Search Report.

Claims 7-8, 12-16 and 19 in part meet the criteria set out in PCT Article 33(2)-(4), because the prior art does not teach or fairly suggest the claimed invention.

----- NEW CITATIONS -----

NONE

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.

PCT/US99/30408

Supplemental Box

(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Boxes I - VIII

Sheet 10

CLASSIFICATION:

The International Patent Classification (IPC) and/or the National classification are as listed below:

IPC(7): C12N 15/12; C07K 14/47, 16/18; C12Q 1/68; A61K 38/17 and US Cl.: 530/300, 350; 536/23.1; 435/252.3, 320.1



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁷ : C12N 15/12, C07K 14/47, C12Q 1/68, A61K 38/17, C07K 16/18		A3	(11) International Publication Number: WO 00/34477
			(43) International Publication Date: 15 June 2000 (15.06.00)
(21) International Application Number: PCT/US99/30408		(72) Inventors; and	
(22) International Filing Date: 10 December 1999 (10.12.99)		(75) Inventors/Applicants (for US only): TANG, Y., Tom [CN/US]; 4230 Ranwick Court, San Jose, CA 95118 (US). YUE, Henry [US/US]; 826 Lois Avenue, Sunnyvale, CA 94087 (US). BAUGHN, Mariah, R. [US/US]; 14244 Santiago Road, San Leandro, CA 94577 (US). HILLMAN, Jennifer, L. [US/US]; 230 Monroe Drive #12, Mountain View, CA 94040 (US). LAL, Preeti [IN/US]; 2382 Lass Drive, Santa Clara, CA 95054 (US). AU-YOUNG, Janice [US/US]; 233 Golden Eagle Lane, Brisbane, CA 94005 (US). YANG, Junming [CN/US]; 7136 Clarendon Street, San Jose, CA 95129 (US). LU, Dyung, Aina, M. [US/US]; 55 Park Belmont Place, San Jose, CA 95136 (US). AZIMZAI, Yalda [US/US]; 2045 Rock Springs Drive, Hayward, CA 94545 (US).	
(30) Priority Data:			
Not furnished	11 December 1998 (11.12.98)	US	
09/210,083	11 December 1998 (11.12.98)	US	
60/119,365	9 February 1999 (09.02.99)	US	
60/124,687	16 March 1999 (16.03.99)	US	
(63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Applications			
US	Not furnished (CIP)		
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US	60/124,687 (CIP)		
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US	09/210,083 (CIP)		
Filed on	11 December 1998 (11.12.98)		
(71) Applicant (for all designated States except US): INCYTE PHARMACEUTICALS, INC. [US/US]; 3174 Porter Drive, Palo Alto, CA 94304 (US).		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).	
		Published With international search report.	
		(88) Date of publication of the international search report: 9 November 2000 (09.11.00)	

(54) Title: NEURON-ASSOCIATED PROTEINS

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1  MA-----GSPSRAAGRRLOLP----- 2417014
1  MEFSLLLPRL E CNGAISAH RNLR LRLP GSSDS GI 3002527

17 -----L L C L F L Q----- 2417014
31 PASASPVAGITGMCTHARLI LY F F L V E M E F GI 3002527

24 ---GATAVLF A V F-----V R Y N H K T 2417014
61 L H V G Q A G L E L P T S D D P S V S A S Q S A R Y R T G H GI 3002527

41 DAAL-----W H----- 2417014
91 H A R L C L A N F C G R N R V S L M C P S W S P E L K Q S T GI 3002527

47 -----R S N H S N A D N E F Y F R Y---P K E S H S 2417014
121 C L S L P K C W D Y R R A A V P G L F I L F F L R H R C P T GI 3002527

68 VAQAGVQRRNLGSLQPSPPR----- 2417014
151 L T Q D E V Q W C D H S S L O P S T E I K H P P A S A S Q GI 3002527

88 -----W-----S F A L V A 2417014
181 V A G T K D M H H Y T W L I F I F I F N F L R Q S L N S V T GI 3002527

```

(57) Abstract

The invention provides human neuron-associated proteins (NEUAP) and polynucleotides which identify and encode NEUAP. The invention also provides expression vectors, host cells, antibodies, agonists, and antagonists. The invention also provides methods for diagnosing, treating, or preventing disorders associated with expression of NEUAP.

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CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 30408

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/12 C07K14/47 C12Q1/68 A61K38/17 C07K16/18

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N C07K C12Q A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 99 06554 A (LACROIX BRUNO ;DUCLERT AYMERIC (FR); GENSET (FR); DUMAS MILNE EDWA) 11 February 1999 (1999-02-11) SEQ ID NO 45,313, ---	1-6,9,10
X	NATIONAL CANCER INSTITUTE, CANCER GENOME ANATOMY PROJECT (CGAP): "Homo sapiens cDNA clone" EMEST DATABASE ENTRY AI022447, ACCESSION NUMBER AI022447, 19 June 1998 (1998-06-19), XP002136735 sequence --- -/--	3-6,9-11



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

Z document member of the same patent family

Date of the actual completion of the international search

2 May 2000

Date of mailing of the international search report

31.08.00

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

ESPEN, J

INTERNATIONAL SEARCH REPORT

International Application No

PC 1/US 30408

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>DATABASE BIOSIS [Online] BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US1996 BUCKEL ALEX ET AL: "Cloning of cDNA encoding human rapsyn and mapping of the RAPSN gene locus to chromosome 11p11.2-p11.1." Database accession no. PREV199699135023 XP002136769 abstract & GENOMICS 1996, vol. 35, no. 3, 1996, pages 613-616, ISSN: 0888-7543 cited in the application -----</p>	

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/US 99/30408

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Although claim 19 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☒ Claims Nos.: 17,18,20
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
See additional sheet, Invention 1.

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 17,18,20

Claims 17 and 18 refer to an agonist/antagonist of the polypeptide of claim 1 without giving a true technical characterization. Moreover, no such compounds are defined in the application. In consequence, the scope of said claims is ambiguous and vague, and their subject-matter is not sufficiently disclosed and supported (Art. 5 and 6, PCT).

No search can be carried out for such purely speculative claims whose wording is, in fact, a mere recitation of the result to be achieved. In addition, claim 20 refers to a method for treating or preventing a disorder comprising administering to a subject the antagonist of claim 18. In consequence, the above comment also applies to claim 20.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

1. Claims: in part: 1-16,19; all as far as applicable

Neuron-associated polypeptide and polynucleotide relating to SEQ ID NOs 1 and 28, and fragments thereof. Expression vector and host cells comprising at least a fragment of such a polynucleotide. Method for detecting such a polynucleotide. Methode for producing such a polypeptide. Pharmaceutical composition comprising such a polypeptide, and method for treating or preventing a disorder by using said composition. Antibody specifically binding with such a polypeptide.

2-27. Claims: in part: 1-16,19; all as far as applicable

as invention 1 but limited to subject-matter relating to SEQ ID NOs 2-27, and 29-54; wherein
invention 2 is limited to SEQ ID NOs 2 and 29
invention 3 is limited to SEQ ID NOs 3 and 30, etc...
invention 27 is limited to SEQ ID NOs 27 and 54.

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INTERNATIONAL SEARCH REPORT

Information on family members

Intern. Appl. No.

PCT/US 99/30408

Patent document
cited in search report

Publication
date

Patent family
member(s)

Publication
date

WO 9906554 A

11-02-1999

AU 8555798 A
EP 1000152 A

22-02-1999
17-05-2000

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
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BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
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CN	China	KR	Republic of Korea	PT	Portugal		
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EE	Estonia	LR	Liberia	SG	Singapore		

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference PF-0637 PCT	FOR FURTHER ACTION see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
International application No. PCT/US 99/ 30408	International filing date (day/month/year) 10/12/1999	(Earliest) Priority Date (day/month/year) 11/12/1998
Applicant INCYTE PHARMACEUTICALS, INC. et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 6 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

- a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

- b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

☒ contained in the international application in written form.

☒ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. ☒ **Certain claims were found unsearchable** (See Box I).

3. ☒ **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No.

☒ as suggested by the applicant.

☐ because the applicant failed to suggest a figure.

☐ because this figure better characterizes the invention.

1A

☐ None of the figures.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 99/30408

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

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3. ☐ Claims Nos.:
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Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

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2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
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See additional sheet, Invention 1.

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 17,18,20

Claims 17 and 18 refer to an agonist/antagonist of the polypeptide of claim 1 without giving a true technical characterization. Moreover, no such compounds are defined in the application. In consequence, the scope of said claims is ambiguous and vague, and their subject-matter is not sufficiently disclosed and supported (Art. 5 and 6, PCT).

No search can be carried out for such purely speculative claims whose wording is, in fact, a mere recitation of the result to be achieved. In addition, claim 20 refers to a method for treating or preventing a disorder comprising administering to a subject the antagonist of claim 18. In consequence, the above comment also applies to claim 20.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

1. Claims: in part: 1-16,19; all as far as applicable

Neuron-associated polypeptide and polynucleotide relating to SEQ ID NOs 1 and 28, and fragments thereof. Expression vector and host cells comprising at least a fragment of such a polynucleotide. Method for detecting such a polynucleotide. Methode for producing such a polypeptide. Pharmaceutical composition comprising such a polypeptide, and method for treating or preventing a disorder by using said composition. Antibody specifically binding with such a polypeptide.

2-27. Claims: in part: 1-16,19; all as far as applicable

as invention 1 but limited to subject-matter relating to SEQ ID NOs 2-27, and 29-54; wherein
invention 2 is limited to SEQ ID NOs 2 and 29
invention 3 is limited to SEQ ID NOs 3 and 30, etc...
invention 27 is limited to SEQ ID NOs 27 and 54.

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/12 C07K14/47 C12Q1/68 A61K38/17 C07K16/18

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N C07K C12Q A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 99 06554 A (LACROIX BRUNO ;DUCLERT AYMERIC (FR); GENSET (FR); DUMAS MILNE EDWA) 11 February 1999 (1999-02-11) SEQ ID NO 45,313, ---	1-6,9,10
X	NATIONAL CANCER INSTITUTE, CANCER GENOME ANATOMY PROJECT (CGAP): "Homo sapiens cDNA clone" EMEST DATABASE ENTRY AI022447, ACCESSION NUMBER AI022447, 19 June 1998 (1998-06-19), XP002136735 sequence --- -/--	3-6,9-11

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

° Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *&* document member of the same patent family

Date of the actual completion of the international search

2 May 2000

Date of mailing of the international search report

31.08.00

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

ESPEN, J

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>DATABASE BIOSIS [Online] BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US1996 BUCKEL ALEX ET AL: "Cloning of cDNA encoding human rapsyn and mapping of the RAPSN gene locus to chromosome 11p11.2-p11.1." Database accession no. PREV199699135023 XP002136769 abstract & GENOMICS 1996, vol. 35, no. 3, 1996, pages 613-616, ISSN: 0888-7543 cited in the application -----</p>	
A		

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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WO 9906554	A	11-02-1999	AU 8555798 A	22-02-1999
			EP 1000152 A	17-05-2000

5' ESTs FOR SECRETED PROTEINS EXPRESSED IN MUSCLE AND OTHER MESODERMAL TISSUES

Background of the Invention

5 The estimated 50,000-100,000 genes scattered along the human chromosomes offer tremendous promise for the understanding, diagnosis, and treatment of human diseases. In addition, probes capable of specifically hybridizing to loci distributed throughout the human genome find applications in the construction of high resolution chromosome maps and in the identification of individuals.

10 In the past, the characterization of even a single human gene was a painstaking process, requiring years of effort. Recent developments in the areas of cloning vectors, DNA sequencing, and computer technology have merged to greatly accelerate the rate at which human genes can be isolated, sequenced, mapped, and characterized. Cloning vectors such as yeast artificial chromosomes (YACs) and bacterial artificial chromosomes (BACs) are able to accept DNA inserts ranging from 300 to 1000 kilobases (kb) or 100-400 kb in length
15 respectively, thereby facilitating the manipulation and ordering of DNA sequences distributed over great distances on the human chromosomes. Automated DNA sequencing machines permit the rapid sequencing of human genes. Bioinformatics software enables the comparison of nucleic acid and protein sequences, thereby assisting in the characterization of human gene products.

20 Currently, two different approaches are being pursued for identifying and characterizing the genes distributed along the human genome. In one approach, large fragments of genomic DNA are isolated, cloned, and sequenced. Potential open reading frames in these genomic sequences are identified using bioinformatics software. However, this approach entails sequencing large stretches of human DNA which do not encode proteins
25 in order to find the protein encoding sequences scattered throughout the genome. In addition to requiring extensive sequencing, the bioinformatics software may mischaracterize the genomic sequences obtained. Thus, the software may produce false positives in which non-coding DNA is mischaracterized as coding DNA or false negatives in which coding DNA is mislabeled as non-coding DNA.

30 An alternative approach takes a more direct route to identifying and characterizing human genes. In this approach, complementary DNAs (cDNAs) are synthesized from

isolated messenger RNAs (mRNAs) which encode human proteins. Using this approach, sequencing is only performed on DNA which is derived from protein coding portions of the genome. Often, only short stretches of the cDNAs are sequenced to obtain sequences called expressed sequence tags (ESTs). The ESTs may then be used to isolate or purify extended
5 cDNAs which include sequences adjacent to the EST sequences. The extended cDNAs may contain all of the sequence of the EST which was used to obtain them or only a portion of the sequence of the EST which was used to obtain them. In addition, the extended cDNAs may contain the full coding sequence of the gene from which the EST was derived or, alternatively, the extended cDNAs may include portions of the coding sequence of the gene
10 from which the EST was derived. It will be appreciated that there may be several extended cDNAs which include the EST sequence as a result of alternate splicing or the activity of alternative promoters.

In the past, these short EST sequences were often obtained from oligo-dT primed cDNA libraries. Accordingly, they mainly corresponded to the 3' untranslated region of the
15 mRNA. In part, the prevalence of EST sequences derived from the 3' end of the mRNA is a result of the fact that typical techniques for obtaining cDNAs are not well suited for isolating cDNA sequences derived from the 5' ends of mRNAs. (Adams *et al.*, *Nature* 377:3-174, 1996; Hillier *et al.*, *Genome Res.* 6:807-828, 1996).

In addition, in those reported instances where longer cDNA sequences have been
20 obtained, the reported sequences typically correspond to coding sequences and do not include the full 5' untranslated region of the mRNA from which the cDNA is derived. Such incomplete sequences may not include the first exon of the mRNA, particularly in situations where the first exon is short. Furthermore, they may not include some exons, often short ones, which are located upstream of splicing sites. Thus, there is a need to obtain sequences
25 derived from the 5' ends of mRNAs.

While many sequences derived from human chromosomes have practical applications, approaches based on the identification and characterization of those chromosomal sequences which encode a protein product are particularly relevant to diagnostic and therapeutic uses. Of the 50,000-100,000 protein coding genes, those genes encoding proteins which are
30 secreted from the cell in which they are synthesized, as well as the secreted proteins themselves, are particularly valuable as potential therapeutic agents. Such proteins are often

involved in cell to cell communication and may be responsible for producing a clinically relevant response in their target cells.

In fact, several secretory proteins, including tissue plasminogen activator, G-CSF, GM-CSF, erythropoietin, human growth hormone, insulin, interferon- α , interferon- β , interferon- γ , and interleukin-2, are currently in clinical use. These proteins are used to treat a wide range of conditions, including acute myocardial infarction, acute ischemic stroke, anemia, diabetes, growth hormone deficiency, hepatitis, kidney carcinoma, chemotherapy induced neutropenia and multiple sclerosis. For these reasons, extended cDNAs encoding secreted proteins or portions thereof represent a particularly valuable source of therapeutic agents. Thus, there is a need for the identification and characterization of secreted proteins and the nucleic acids encoding them.

In addition to being therapeutically useful themselves, secretory proteins include short peptides, called signal peptides, at their amino termini which direct their secretion. These signal peptides are encoded by the signal sequences located at the 5' ends of the coding sequences of genes encoding secreted proteins. Because these signal peptides will direct the extracellular secretion of any protein to which they are operably linked, the signal sequences may be exploited to direct the efficient secretion of any protein by operably linking the signal sequences to a gene encoding the protein for which secretion is desired. In addition, portions of signal sequences may also be used to direct the intracellular import of a peptide or protein of interest. This may prove beneficial in gene therapy strategies in which it is desired to deliver a particular gene product to cells other than the cell in which it is produced. Signal sequences encoding signal peptides also find application in simplifying protein purification techniques. In such applications, the extracellular secretion of the desired protein greatly facilitates purification by reducing the number of undesired proteins from which the desired protein must be selected. Thus, there exists a need to identify and characterize the 5' portions of the genes for secretory proteins which encode signal peptides.

Public information on the number of human genes for which the promoters and upstream regulatory regions have been identified and characterized is quite limited. In part, this may be due to the difficulty of isolating such regulatory sequences. Upstream regulatory sequences such as transcription factor binding sites are typically too short to be utilized as probes for isolating promoters from human genomic libraries. Recently, some approaches

have been developed to isolate human promoters. One of them consists of making a CpG island library (Cross, *et al.*, *Nature Genetics* 6: 236-244, 1994). The second consists of isolating human genomic DNA sequences containing SpeI binding sites by the use of SpeI binding protein. (Mortlock *et al.*, *Genome Res.* 6:327-335, 1996). Both of these approaches
5 have their limits due to a lack of specificity or of comprehensiveness.

The present 5' ESTs may be used to efficiently identify and isolate upstream regulatory regions which control the location, developmental stage, rate, and quantity of protein synthesis, as well as the stability of the mRNA. (Theil, *BioFactors* 4:87-93, 1993). Once identified and characterized, these regulatory regions may be utilized in gene therapy or
10 protein purification schemes to obtain the desired amount and locations of protein synthesis or to inhibit, reduce, or prevent the synthesis of undesirable gene products.

In addition, ESTs containing the 5' ends of secretory protein genes may include sequences useful as probes for chromosome mapping and the identification of individuals. Thus, there is a need to identify and characterize the sequences upstream of the 5' coding
15 sequences of genes encoding secretory proteins.

Summary of the Invention

The present invention relates to purified, isolated, or recombinant ESTs which include sequences derived from the authentic 5' ends of their corresponding mRNAs. The term
20 "corresponding mRNA" refers to the mRNA which was the template for the cDNA synthesis which produced the 5' EST. These sequences will be referred to hereinafter as "5' ESTs." As used herein, the term "purified" does not require absolute purity; rather, it is intended as a relative definition. Individual 5' EST clones isolated from a cDNA library have been conventionally purified to electrophoretic homogeneity. The sequences obtained from these
25 clones could not be obtained directly either from the library or from total human DNA. The cDNA clones are not naturally occurring as such, but rather are obtained via manipulation of a partially purified naturally occurring substance (messenger RNA). The conversion of mRNA into a cDNA library involves the creation of a synthetic substance (cDNA) and pure individual cDNA clones can be isolated from the synthetic library by clonal selection. Thus,
30 creating a cDNA library from messenger RNA and subsequently isolating individual clones from that library results in an approximately 10^4 - 10^6 fold purification of the native message.

Purification of starting material or natural material to at least one order of magnitude, preferably two or three orders, and more preferably four or five orders of magnitude is expressly contemplated.

As used herein, the term "isolated" requires that the material be removed from its original environment (e.g., the natural environment if it is naturally occurring). For example, a naturally-occurring polynucleotide present in a living animal is not isolated, but the same polynucleotide, separated from some or all of the coexisting materials in the natural system, is isolated.

As used herein, the term "recombinant" means that the 5' EST is adjacent to "backbone" nucleic acid to which it is not adjacent in its natural environment. Additionally, to be "enriched" the 5' ESTs will represent 5% or more of the number of nucleic acid inserts in a population of nucleic acid backbone molecules. Backbone molecules according to the present invention include nucleic acids such as expression vectors, self-replicating nucleic acids, viruses, integrating nucleic acids, and other vectors or nucleic acids used to maintain or manipulate a nucleic acid insert of interest. Preferably, the enriched 5' ESTs represent 15% or more of the number of nucleic acid inserts in the population of recombinant backbone molecules. More preferably, the enriched 5' ESTs represent 50% or more of the number of nucleic acid inserts in the population of recombinant backbone molecules. In a highly preferred embodiment, the enriched 5' ESTs represent 90% or more of the number of nucleic acid inserts in the population of recombinant backbone molecules.

"Stringent", moderate," and "low" hybridization conditions are as defined in Example 29.

Unless otherwise indicated, a "complementary" sequence is fully complementary.

Thus, 5' ESTs in cDNA libraries in which one or more 5' ESTs make up 5% or more of the number of nucleic acid inserts in the backbone molecules are "enriched recombinant 5' ESTs" as defined herein. Likewise, 5' ESTs in a population of plasmids in which one or more 5' EST of the present invention have been inserted such that they represent 5% or more of the number of inserts in the plasmid backbone are "enriched recombinant 5' ESTs" as defined herein. However, 5' ESTs in cDNA libraries in which 5' ESTs constitute less than 5% of the number of nucleic acid inserts in the population of backbone molecules, such as libraries in

which backbone molecules having a 5' EST insert are extremely rare, are not "enriched recombinant 5' ESTs."

In particular, the present invention relates to 5' ESTs which are derived from genes encoding secreted proteins. As used herein, a "secreted" protein is one which, when
5 expressed in a suitable host cell, is transported across or through a membrane, including transport as a result of signal peptides in its amino acid sequence. "Secreted" proteins include without limitation proteins secreted wholly (e.g. soluble proteins), or partially (e.g. receptors) from the cell in which they are expressed. "Secreted" proteins also include without limitation proteins which are transported across the membrane of the endoplasmic reticulum.

10 Such 5' ESTs include nucleic acid sequences, called signal sequences, which encode signal peptides which direct the extracellular secretion of the proteins encoded by the genes from which the 5' ESTs are derived. Generally, the signal peptides are located at the amino termini of secreted proteins.

Secreted proteins are translated by ribosomes associated with the "rough"
15 endoplasmic reticulum. Generally, secreted proteins are co-translationally transferred to the membrane of the endoplasmic reticulum. Association of the ribosome with the endoplasmic reticulum during translation of secreted proteins is mediated by the signal peptide. The signal peptide is typically cleaved following its co-translational entry into the endoplasmic reticulum. After delivery to the endoplasmic reticulum, secreted proteins may proceed through the
20 Golgi apparatus. In the Golgi apparatus, the proteins may undergo post-translational modification before entering secretory vesicles which transport them across the cell membrane.

The 5' ESTs of the present invention have several important applications. For example, they may be used to obtain and express cDNA clones which include the full protein
25 coding sequences of the corresponding gene products, including the authentic translation start sites derived from the 5' ends of the coding sequences of the mRNAs from which the 5' ESTs are derived. These cDNAs will be referred to hereinafter as "full length cDNAs." These cDNAs may also include DNA derived from mRNA sequences upstream of the translation start site. The full length cDNA sequences may be used to express the proteins
30 corresponding to the 5' ESTs. As discussed above, secreted proteins are therapeutically important. Thus, the proteins expressed from the cDNAs may be useful in treating or

controlling a variety of human conditions. The 5' ESTs may also be used to obtain the corresponding genomic DNA. The term "corresponding genomic DNA" refers to the genomic DNA which encodes the mRNA from which the 5' EST was derived.

Alternatively, the 5' ESTs may be used to obtain and express extended cDNAs
5 encoding portions of the secreted protein. The portions may comprise the signal peptides of the secreted proteins or the mature proteins generated when the signal peptide is cleaved off. The portions may also comprise polypeptides having at least 10 consecutive amino acids encoded by the extended cDNAs or full length cDNAs. Alternatively, the portions may comprise at least 15 consecutive amino acids encoded by the extended cDNAs or full length
10 cDNAs. In some embodiments, the portions may comprise at least 25 consecutive amino acids encoded by the extended cDNAs or full length cDNAs. In other embodiments, the portions may comprise at least 40 amino acids encoded by the extended cDNAs or full length cDNAs.

Antibodies which specifically recognize the entire secreted proteins encoded by the
15 extended cDNAs, full length cDNAs, or fragments thereof having at least 10 consecutive amino acids, at least 15 consecutive amino acids, at least 25 consecutive amino acids, or at least 40 consecutive amino acids may also be obtained as described below. Antibodies which specifically recognize the mature protein generated when the signal peptide is cleaved may also be obtained as described below. Similarly, antibodies which specifically recognize the
20 signal peptides encoded by the extended cDNAs or full length cDNAs may also be obtained.

In some embodiments, the extended cDNAs obtained using the 5' ESTs include the signal sequence. In other embodiments, the extended cDNAs obtained using the 5' ESTs may include the full coding sequence for the mature protein (*i.e.* the protein generated when the signal polypeptide is cleaved off). In addition, the extended cDNAs obtained using the 5'
25 ESTs may include regulatory regions upstream of the translation start site or downstream of the stop codon which control the amount, location, or developmental stage of gene expression.

As discussed above, secreted proteins are therapeutically important. Thus, the proteins expressed from the extended cDNAs or full length cDNAs obtained using the 5'
30 ESTs may be useful in treating or controlling a variety of human conditions.

The 5' ESTs (or cDNAs or genomic DNAs obtained therefrom) may be used in forensic procedures to identify individuals or in diagnostic procedures to identify individuals having genetic diseases resulting from abnormal expression of the genes corresponding to the 5' ESTs. In addition, the present invention is useful for constructing a high resolution map of the human chromosomes.

The present invention also relates to secretion vectors capable of directing the secretion of a protein of interest. Such vectors may be used in gene therapy strategies in which it is desired to produce a gene product in one cell which is to be delivered to another location in the body. Secretion vectors may also facilitate the purification of desired proteins.

The present invention also relates to expression vectors capable of directing the expression of an inserted gene in a desired spatial or temporal manner or at a desired level. Such vectors may include sequences upstream of the 5' ESTs, such as promoters or upstream regulatory sequences.

Finally, the present invention may also be used for gene therapy to control or treat genetic diseases. Signal peptides may also be fused to heterologous proteins to direct their extracellular secretion.

Bacterial clones containing Bluescript plasmids having inserts containing the 5' ESTs of the present invention (SEQ ID NOs: 38-305 are presently stored at 80°C in 4% (v/v) glycerol in the inventor's laboratories under the designations listed next to the SEQ ID NOs in II). The inserts may be recovered from the deposited materials by growing the appropriate clones on a suitable medium. The Bluescript DNA can then be isolated using plasmid isolation procedures familiar to those skilled in the art such as alkaline lysis minipreps or large scale alkaline lysis plasmid isolation procedures. If desired the plasmid DNA may be further enriched by centrifugation on a cesium chloride gradient, size exclusion chromatography, or anion exchange chromatography. The plasmid DNA obtained using these procedures may then be manipulated using standard cloning techniques familiar to those skilled in the art. Alternatively, a PCR can be done with primers designed at both ends of the EST insertion. The PCR product which corresponds to the 5' EST can then be manipulated using standard cloning techniques familiar to those skilled in the art.

One aspect of the present invention is a purified or isolated nucleic acid having the sequence of one of SEQ ID NOs: 38-305 or having a sequence complementary thereto. In one embodiment, the nucleic acid is recombinant.

5 Another aspect of the present invention is a purified or isolated nucleic acid comprising at least 10 consecutive bases of the sequence of one of SEQ ID NOs: 38-305 or one of the sequences complementary thereto.

Yet another aspect of the present invention is a purified or isolated nucleic acid comprising at least 15 consecutive bases of one of the sequences of SEQ ID NOs: 38-305 or one of the sequences complementary thereto. In one embodiment, the nucleic acid is
10 recombinant.

A further aspect of the present invention is a purified or isolated nucleic acid of at least 15 bases capable of hybridizing under stringent conditions to the sequence of one of SEQ ID NOs: 38-305 or one of the sequences complementary to the sequences of SEQ ID NOs: 38-305. In one embodiment, the nucleic acid is recombinant.

15 Another aspect of the present invention is a purified or isolated nucleic acid encoding a human gene product, said human gene product having a sequence partially encoded by one of the sequences of SEQ ID NO: 38-305.

Still another aspect of the present invention is a method of making a cDNA encoding a human secretory protein, said human secretory protein being partially encoded by one of
20 SEQ ID NOs 38-305, comprising the steps of contacting a collection of mRNA molecules from human cells with a primer comprising at least 15 consecutive nucleotides of a sequence complementary to one of SEQ ID NOs: 38-305; hybridizing said primer to an mRNA in said collection that encodes said protein; reverse transcribing said hybridized primer to make a first cDNA strand from said mRNA; making a second cDNA strand complementary to said first
25 cDNA strand; and isolating the resulting cDNA encoding said protein comprising said first cDNA strand and said second cDNA strand.

Another aspect of the invention is an isolated or purified cDNA encoding a human secretory protein, said human secretory protein comprising the protein encoded by one of SEQ ID NOs 38-305 or a fragment thereof of at least 10 amino acids, said cDNA being
30 obtainable by the method described in the preceding paragraph. In one embodiment, the

cDNA comprises the full protein coding sequence of said protein which sequence is partially included in one of the sequences of SEQ ID NOs: 38-305.

Another aspect of the present invention is a method of making a cDNA encoding a human secretory protein that is partially encoded by one of SEQ ID NOs 38-305, comprising
5 the steps of obtaining a cDNA comprising one of the sequences of SEQ ID NOs: 38-305; contacting said cDNA with a detectable probe comprising at least 15 consecutive nucleotides of said sequence of SEQ ID NO: 38-305 or a sequence complementary thereto under conditions which permit said probe to hybridize to said cDNA; identifying a cDNA which hybridizes to said detectable probe; and isolating said cDNA which hybridizes to said probe.

10 Another aspect of the present invention is an isolated or purified cDNA encoding a human secretory protein, said human secretory protein comprising the protein encoded by one of SEQ ID NOs 38-305 or a fragment thereof of at least 10 amino acids, said cDNA being obtainable by the method described in the preceding paragraph. In one embodiment, the cDNA comprises the full protein coding sequence partially included in one of the
15 sequences of SEQ ID NOs: 38-305.

Another aspect of the present invention is a method of making a cDNA comprising one of the sequence of SEQ ID NOs: 38-305, comprising the steps of contacting a collection of mRNA molecules from human cells with a first primer capable of hybridizing to the polyA tail of said mRNA; hybridizing said first primer to said polyA tail; reverse transcribing said
20 mRNA to make a first cDNA strand; making a second cDNA strand complementary to said first cDNA strand using at least one primer comprising at least 15 nucleotides of one of the sequences of SEQ ID NOs 38-305; and isolating the resulting cDNA comprising said first cDNA strand and said second cDNA strand.

Another aspect of the present invention is an isolated or purified cDNA encoding a
25 human secretory protein, said human secretory protein comprising the protein encoded by one of SEQ ID NOs 38-305 or a fragment thereof of at least 10 amino acids, said cDNA being obtainable by the method described in the preceding paragraph. In one embodiment, the cDNA comprises the full protein coding sequence partially included in one of the sequences of SEQ ID NOs: 38-305.

30 In one embodiment of the method described in the two paragraphs above, the second cDNA strand is made by contacting said first cDNA strand with a first pair of primers, said

first pair of primers comprising a second primer comprising at least 15 consecutive nucleotides of one of the sequences of SEQ ID NOs 38-305 and a third primer having a sequence therein which is included within the sequence of said first primer; performing a first polymerase chain reaction with said first pair of nested primers to generate a first PCR product; contacting said first PCR product with a second pair of primers, said second pair of primers comprising a fourth primer, said fourth primer comprising at least 15 consecutive nucleotides of said sequence of one of SEQ ID NOs: 38-305, and a fifth primer, said fourth and fifth primers being capable of hybridizing to sequences within said first PCR product; and performing a second polymerase chain reaction, thereby generating a second PCR product.

One aspect of the present invention is an isolated or purified cDNA encoding a human secretory protein, said human secretory protein comprising the protein encoded by one of SEQ ID NOs 38-305, or a fragment thereof of at least 10 amino acids, said cDNA being obtainable by the method of the preceding paragraph. In one embodiment, the cDNA comprises the full protein coding sequence partially included in one of the sequences of SEQ ID NOs: 38-305.

Another aspect of the present invention is the method described four paragraphs above in which the second cDNA strand is made by contacting said first cDNA strand with a second primer comprising at least 15 consecutive nucleotides of the sequences of SEQ ID NOs: 38-305; hybridizing said second primer to said first strand cDNA; and extending said hybridized second primer to generate said second cDNA strand.

Another aspect of the present invention is an isolated or purified cDNA encoding a human secretory protein, said human secretory protein comprising the protein partially encoded by one of SEQ ID NOs 38-305 or comprising a fragment thereof of at least 10 amino acids, said cDNA being obtainable by the method described in the preceding paragraph. In one embodiment, the cDNA comprises the full protein coding sequence partially included in one of the sequences of SEQ ID NOs: 38-305.

Another aspect of the present invention is a method of making a protein comprising one of the sequences of SEQ ID NOs: 306-573, comprising the steps of obtaining a cDNA encoding the full protein sequence partially included in one of the sequences of sequence of SEQ ID NOs: 38-305; inserting said cDNA in an expression vector such that said cDNA is

operably linked to a promoter; introducing said expression vector into a host cell whereby said host cell produces the protein encoded by said cDNA; and isolating said protein.

Another aspect of the present invention is an isolated protein obtainable by the method described in the preceding paragraph.

5 Another aspect of the present invention is a method of obtaining a promoter DNA comprising the steps of obtaining DNAs located upstream of the nucleic acids of SEQ ID NOs: 38-305 or the sequences complementary thereto; screening said upstream DNAs to identify a promoter capable of directing transcription initiation; and isolating said DNA comprising said identified promoter. In one embodiment, the obtaining step comprises
10 chromosome walking from said nucleic acids of SEQ ID NOs: 38-305 or sequences complementary thereto. In another embodiment, the screening step comprises inserting said upstream sequences into a promoter reporter vector. In another embodiment, the screening step comprises identifying motifs in said upstream DNAs which are transcription factor binding sites or transcription start sites.

15 Another aspect of the present invention is an isolated promoter obtainable by the method described above.

Another aspect of the present invention is an isolated or purified protein comprising one of the sequences of SEQ ID NOs: 306-573.

Another aspect of the present invention is the inclusion of at least one of the
20 sequences of SEQ ID NOs: 38-305, or one of the sequences complementary to the sequences of SEQ ID NOs: 38-305, or a fragment thereof of at least 15 consecutive nucleotides in an array of discrete ESTs or fragments thereof of at least 15 nucleotides in length. In one embodiment, the array includes at least two of the sequences of SEQ ID NOs: 38-305, the sequences complementary to the sequences of SEQ ID NOs: 38-305, or fragments thereof of
25 at least 15 consecutive nucleotides. In another embodiment, the array includes at least five of the sequences of SEQ ID NOs: 38-305, the sequences complementary to the sequences of SEQ ID NOs: 38-305, or fragments thereof of at least 15 consecutive nucleotides.

Another aspect of the present invention is a promoter having a sequence selected from the group consisting of SEQ ID NOs: 31, 34, and 37.

Brief Description of the Drawings

Figure 1 is a summary of a procedure for obtaining cDNAs which have been selected to include the 5' ends of the mRNAs from which they derived.

Figure 2 shows the distribution of Von Heijne scores for 5' ESTs in each of the categories described herein and the probability that these 5' ESTs encode a signal peptide.

Figure 3 summarizes a general method used to clone and sequence extended cDNAs containing sequences adjacent to 5' ESTs.

Figure 4 (description of promoters structure isolated from SignalTag 5' ESTs) provides a schematic description of promoters isolated and the way they are assembled with the corresponding 5' tags.

Detailed Description of the Preferred Embodiment

Table IV is an analysis of the 43 amino acids located at the N terminus of all human SwissProt proteins to determine the frequency of false positives and false negatives using the techniques for signal peptide identification described herein.

Table V shows the distribution of 5' ESTs in each category described herein and the number of 5' ESTs in each category having a given minimum Von Heijne's score.

Table VI shows the distribution of 5' ESTs in each category described herein with respect to the tissue from which the 5' ESTs of the corresponding mRNA were obtained.

Table VII describes the transcription factor binding sites present in each of these promoters.

I. General Methods for Obtaining 5' ESTs derived from mRNAs with intact 5' ends

In order to obtain the 5' ESTs of the present invention, mRNAs with intact 5' ends must be obtained. Currently, there are two approaches for obtaining such mRNAs with intact 5' ends as described below: either chemical (1) or enzymatic (2).

1. Chemical Methods for Obtaining mRNAs having Intact 5' Ends

One of these approaches is a chemical modification method involving derivatization of the 5' ends of the mRNAs and selection of the derivatized mRNAs. The 5' ends of eukaryotic mRNAs possess a structure referred to as a "cap" which comprises a guanosine

methyated at the 7 position. The cap is joined to the first transcribed base of the mRNA by a 5', 5'-triphosphate bond. In some instances, the 5' guanosine is methylated in both the 2 and 7 positions. Rarely, the 5' guanosine is trimethylated at the 2, 7 and 7 positions. In the chemical method for obtaining mRNAs having intact 5' ends, the 5' cap is specifically
5 derivatized and coupled to a reactive group on an immobilizing substrate. This specific derivatization is based on the fact that only the ribose linked to the methylated guanosine at the 5' end of the mRNA and the ribose linked to the base at the 3' terminus of the mRNA, possess 2', 3'-cis diols.

Optionally, the 2', 3'-cis diol of the 3' terminal ribose may be chemically
10 modified, substituted, converted, or eliminated, leaving only the ribose linked to the methylated guanosine at the 5' end of the mRNA with a 2', 3'-cis diol. A variety of techniques are available for eliminating the 2', 3'-cis diol on the 3' terminal ribose. For example, controlled alkaline hydrolysis may be used to generate mRNA fragments in which the 3' terminal ribose is a 3'-phosphate, 2'-phosphate or (2', 3')-cyclophosphate.
15 Thereafter, the fragment which includes the original 3' ribose may be eliminated from the mixture through chromatography on an oligodT column. Alternatively, a base which lacks the 2', 3'-cis diol may be added to the 3' end of the mRNA using an RNA ligase such as T4 RNA ligase. Example 1 below describes a method for ligation of a nucleoside diphosphate to the 3' end of messenger RNA.

20

EXAMPLE 1

Ligation of the Nucleoside Diphosphate pCp to the 3' End of mRNA

One μg of RNA was incubated in a final reaction medium of 10 μl in the presence of 5 U of T₄ phage RNA ligase in the buffer provided by the manufacturer (Gibco -
25 BRL), 40 U of the RNase inhibitor RNasin (Promega) and, 2 μl of ³²pCp (Amersham #PB 10208). The incubation was performed at 37°C for 2 hours or overnight at 7-8°C.

Following modification or elimination of the 2', 3'-cis diol at the 3' ribose, the 2', 3'-cis diol present at the 5' end of the mRNA may be oxidized using reagents such as NaBH₄,
30 NaBH₃CN, or sodium periodate, thereby converting the 2', 3'-cis diol to a dialdehyde.

Example 2 describes the oxidation of the 2', 3'-cis diol at the 5' end of the mRNA with sodium periodate.

EXAMPLE 2

5 Oxidation of 2', 3'-cis diol at the 5' End of the mRNA with Sodium Periodate

0.1 OD unit of either a capped oligoribonucleotide of 47 nucleotides (including the cap) or an uncapped oligoribonucleotide of 46 nucleotides were treated as follows. The oligoribonucleotides were produced by *in vitro* transcription using the transcription kit "AmpliScribe T7" (Epicentre Technologies). As indicated below, the DNA template for the
 10 RNA transcript contained a single cytosine. To synthesize the uncapped RNA, all four NTPs were included in the *in vitro* transcription reaction. To obtain the capped RNA, GTP was replaced by an analogue of the cap, m7G(5')ppp(5')G. This compound, recognized by the polymerase, was incorporated into the 5' end of the nascent transcript during the initiation of transcription but was not incorporated during the extension step. Consequently, the resulting
 15 RNA contained a cap at its 5' end. The sequences of the oligoribonucleotides produced by the *in vitro* transcription reaction were:

+Cap:

5'm7GpppGCAUCCUACUCCCAUCCAAUUCCACCCUAACUCCUCCCAUCUCCAC-
 3' (SEQ ID NO:1)

20 -Cap:

5'-pppGCAUCCUACUCCCAUCCAAUUCCACCCUAACUCCUCCCAUCUCCAC-3'
 (SEQ ID NO:2)

The oligoribonucleotides were dissolved in 9 µl of acetate buffer (0.1 M sodium acetate, pH 5.2) and 3 µl of freshly prepared 0.1 M sodium periodate solution. The mixture
 25 was incubated for 1 hour in the dark at 4°C or room temperature. Thereafter, the reaction was stopped by adding 4 µl of 10% ethylene glycol. The product was ethanol precipitated, resuspended in at least 10 µl of water or appropriate buffer and dialyzed against water.

The resulting aldehyde groups may then be coupled to molecules having a reactive
 30 amine group, such as hydrazine, carbazide, thiocarbazide or semicarbazide groups, in order to facilitate enrichment of the 5' ends of the mRNAs. Molecules having reactive amine groups

which are suitable for use in selecting mRNAs having intact 5' ends include avidin, proteins, antibodies, vitamins, ligands capable of specifically binding to receptor molecules, or oligonucleotides. Example 3 below describes the coupling of the resulting dialdehyde to biotin.

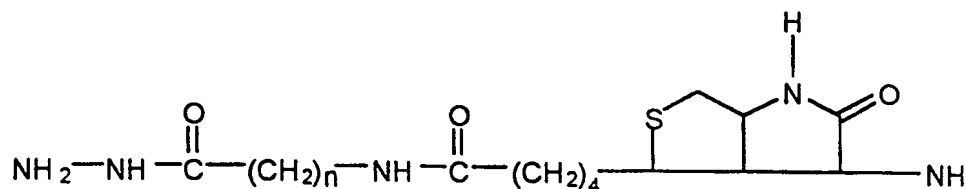
5

EXAMPLE 3

Coupling of the Dialdehyde at the 5' End of Transcripts with Biotin

The oxidation product obtained in Example 2 was dissolved in 50 μ l of sodium acetate at a pH between 5 and 5.2 and 50 μ l of freshly prepared 0.02 M solution of biotin hydrazide in a methoxyethanol/water mixture (1:1) of formula:

10



In the compound used in these experiments, $n=5$. However, it will be appreciated that other commercially available hydrazides may also be used, such as molecules of the above formula in which n varies from 0 to 5. The mixture was then incubated for 2 hours at 37°C, precipitated with ethanol and dialyzed against distilled water. Example 4 demonstrates the specificity of the biotinylation reaction.

15

EXAMPLE 4

20

Specificity of Biotinylation of Capped Transcripts

The specificity of the biotinylation for capped mRNAs was evaluated by gel electrophoresis of the following samples:

Sample 1. The 46 nucleotide uncapped *in vitro* transcript prepared as in Example 2 and labeled with 32 Pcp as described in Example 1.

25

Sample 2. The 46 nucleotide uncapped *in vitro* transcript prepared as in Example 2, labeled with 32 Pcp as described in Example 1, treated with the oxidation reaction of Example 2, and subjected to the biotinylation conditions of Example 3.

Sample 3. The 47 nucleotide capped *in vitro* transcript prepared as in Example 2 and labeled with ^{32}pCp as described in Example 1.

Sample 4. The 47 nucleotide capped *in vitro* transcript prepared as in Example 2, labeled with ^{32}pCp as described in Example 1, treated with the oxidation reaction of Example 2, and subjected to the biotinylation conditions of Example 3.

Samples 1 and 2 had identical migration rates, demonstrating that the uncapped RNAs were not oxidized and biotinylated. Sample 3 migrated more slowly than Samples 1 and 2, while Sample 4 exhibited the slowest migration. The difference in migration of the RNAs in Samples 3 and 4 demonstrates that the capped RNAs were specifically biotinylated.

In some cases, mRNAs having intact 5' ends may be enriched by binding the molecule containing a reactive amine group to a suitable solid phase substrate such as the inside of the vessel containing the mRNAs, magnetic beads, chromatography matrices, or nylon or nitrocellulose membranes. For example, where the molecule having a reactive amine group is biotin, the solid phase substrate may be coupled to avidin or streptavidin. Alternatively, where the molecule having the reactive amine group is an antibody or receptor ligand, the solid phase substrate may be coupled to the cognate antigen or receptor. Finally, where the molecule having a reactive amine group comprises an oligonucleotide, the solid phase substrate may comprise a complementary oligonucleotide.

The mRNAs having intact 5' ends may be released from the solid phase following the enrichment procedure. For example, where the dialdehyde is coupled to biotin hydrazide and the solid phase comprises streptavidin, the mRNAs may be released from the solid phase by simply heating to 95 degrees Celsius in 2% SDS. In some methods, the molecule having a reactive amine group may also be cleaved from the mRNAs having intact 5' ends following enrichment. Example 5 describes the capture of biotinylated mRNAs with streptavidin coated beads and the release of the biotinylated mRNAs from the beads following enrichment.

EXAMPLE 5

Capture and Release of Biotinylated mRNAs Using Streptavidin Coated Beads

The streptavidin coated magnetic beads were prepared according to the manufacturer's instructions (CPG Inc., USA). The biotinylated mRNAs were added to a

hybridization buffer (1.5 M NaCl, pH 5 - 6). After incubating for 30 minutes, the unbound and nonbiotinylated material was removed. The beads were then washed several times in water with 1% SDS. The beads thus obtained were incubated for 15 minutes at 95°C in water containing 2% SDS.

- 5 Example 6 demonstrates the efficiency with which biotinylated mRNAs were recovered from the streptavidin coated beads.

EXAMPLE 6

Efficiency of Recovery of Biotinylated mRNAs

- 10 The efficiency of the recovery procedure was evaluated as follows. Capped RNAs were labeled with ³²pCp, oxidized, biotinylated and bound to streptavidin coated beads as described above. Subsequently, the bound RNAs were incubated for 5, 15 or 30 minutes at 95°C in the presence of 2% SDS.

- 15 The products of the reaction were analyzed by electrophoresis on 12% polyacrylamide gels under denaturing conditions (7 M urea). The gels were subjected to autoradiography. During this manipulation, the hydrazone bonds were not reduced.

Increasing amounts of nucleic acids were recovered as incubation times in 2% SDS increased, demonstrating that biotinylated mRNAs were efficiently recovered.

- 20 In an alternative method for obtaining mRNAs having intact 5' ends, an oligonucleotide which has been derivatized to contain a reactive amine group is specifically coupled to mRNAs having an intact cap. Preferably, the 3' end of the mRNA is blocked prior to the step in which the aldehyde groups are joined to the derivatized oligonucleotide, as described above, so as to prevent the derivatized oligonucleotide from being joined to the 3'
- 25 end of the mRNA. For example, pCp may be attached to the 3' end of the mRNA using T4 RNA ligase as described in example 1. However, as discussed above, blocking the 3' end of the mRNA is an optional step. Derivatized oligonucleotides may be prepared as described in Example 7.

EXAMPLE 7Derivatization of Oligonucleotides

An oligonucleotide phosphorylated at its 3' end was converted to a 3' hydrazide in 3' by treatment with an aqueous solution of hydrazine or of dihydrazide of the formula
5 $H_2N(R1)NH_2$ at about 1 to 3 M, and at pH 4.5 at a temperature of 8°C overnight. This incubation was performed in the presence of a carbodiimide type agent soluble in water such as 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide at a final concentration of 0.3 M.

The derivatized oligonucleotide was then separated from the other agents and products using a standard technique for isolating oligonucleotides.

10 As discussed above, the mRNAs to be enriched may be treated to eliminate the 3' OH groups which may be present thereon. This may be accomplished by enzymatic ligation of sequences lacking a 3' OH, such as pCp, as described in Example 1. Alternatively, the 3' OH groups may be eliminated by alkaline hydrolysis as described in Example 8 below.

15

EXAMPLE 8Elimination of 3' OH Groups of mRNA Using Alkaline Hydrolysis

In a total volume of 100 µl of 0.1 N sodium hydroxide, 1.5 µg mRNA is incubated for 40 to 60 minutes at 4°C. The solution is neutralized with acetic acid and precipitated with ethanol.

20 Following the optional elimination of the 3' OH groups, the diol groups at the 5' ends of the mRNAs are oxidized as described below in Example 9.

EXAMPLE 9Oxidation of Diols of mRNA

25 Up to 1 OD unit of RNA was dissolved in 9 µl of buffer (0.1 M sodium acetate, pH 6-7) or water and 3 µl of freshly prepared 0.1 M sodium periodate solution. The reaction was incubated for 1 h in the dark at 4°C or room temperature. Following the incubation, the reaction was stopped by adding 4 µl of 10% ethylene glycol. Thereafter the mixture was incubated at room temperature for 15 minutes. After ethanol precipitation, the product was
30 resuspended in at least 10 µl of water or appropriate buffer and dialyzed against water.

Following oxidation of the diol groups at the 5' ends of the mRNAs, the derivatized oligonucleotide was joined to the resulting aldehydes as described in Example 10.

EXAMPLE 10

5 Ligature of Aldehydes of mRNA to Derivatized Oligonucleotides

The oxidized mRNA was dissolved in an acidic medium such as 50 µl of sodium acetate pH 4-6. Fifty µl of a solution of the derivatized oligonucleotide were added in order to obtain an mRNA:derivatized oligonucleotide ratio of 1:20. The mixture was reduced with a borohydride and incubated for 2 h at 37°C or overnight (14 h) at 10°C. The mixture was
10 then ethanol precipitated, resuspended in 10 µl or more of water or appropriate buffer and dialyzed against distilled water. If desired, the resulting product may be analyzed using acrylamide gel electrophoresis, HPLC analysis, or other conventional techniques.

Following the attachment of the derivatized oligonucleotide to the mRNAs, a reverse
15 transcription reaction may be performed as described in Example 11 below.

EXAMPLE 11

Reverse Transcription of mRNAs Ligatured to Derivatized Oligonucleotides

An oligodeoxyribonucleotide was derivatized as follows. Three OD units of an
20 oligodeoxyribonucleotide of sequence 5'ATCAAGAATTCGCACGAGACCATTAA3' (SEQ ID NO:3) having 5'-OH and 3'-P ends were dissolved in 70 µl of a 1.5 M hydroxybenzotriazole solution, pH 5.3, prepared in dimethylformamide/water (75:25) containing 2 µg of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide. The mixture was incubated for 2 h 30 min at 22°C and then precipitated twice in LiClO₄/acetone. The pellet
25 was resuspended in 200 µl of 0.25 M hydrazine and incubated at 8°C from 3 to 14 h. Following the hydrazine reaction, the mixture was precipitated twice in LiClO₄/acetone.

The messenger RNAs to be reverse transcribed were extracted from blocks of placenta having sides of 2 cm which had been stored at -80°C. The total RNA was extracted using conventional acidic phenol techniques. Oligo-dT chromatography was used to purify
30 the mRNAs. The integrity of the mRNAs was checked by Northern-blotting.

The diol groups on 7 μ g of the placental mRNAs were oxidized as described above in Example 9. The derivatized oligonucleotide was joined to the mRNAs as described in Example 10 above except that the precipitation step was replaced by an exclusion chromatography step to remove derivatized oligodeoxyribonucleotides which were not joined to mRNAs. Exclusion chromatography was performed as follows:

Ten ml of Ultrogel AcA34 (BioSeptra#230151) gel, a mix of agarose and acrylamide, were equilibrated in 50 ml of a solution of 10 mM Tris pH 8.0, 300 mM NaCl, 1 mM EDTA, and 0.05% SDS. The mixture was allowed to sediment. The supernatant was eliminated and the gel was resuspended in 50 ml of buffer. This procedure was repeated 2 or 3 times.

A glass bead (diameter 3 mm) was introduced into a 2 ml disposable pipette (length 25 cm). The pipette was filled with the gel suspension until the height of the gel stabilized at 1 cm from the top of the pipette. The column was then equilibrated with 20 ml of equilibration buffer (10 mM Tris HCl pH 7.4, 20 mM NaCl).

Ten μ l of the mRNA which had reacted with the derivatized oligonucleotide were mixed in 39 μ l of 10 mM urea and 2 μ l of blue-glycerol buffer, which had been prepared by dissolving 5 mg of bromophenol blue in 60% glycerol (v/v), and passing the mixture through a 0.45 μ m diameter filter.

The column was then loaded with the mRNAs coupled to the oligonucleotide. As soon as the sample had penetrated, equilibration buffer was added. Hundred μ l fractions were then collected. Derivatized oligonucleotide which had not been attached to mRNA appeared in fraction 16 and later fractions. Thus, fractions 3 to 15 were combined and precipitated with ethanol.

To determine whether the derivatized oligonucleotide was actually linked to mRNA, one tenth of the combined fractions were spotted twice on a nylon membrane and hybridized to a radioactive probe using conventional techniques. The 32 P labeled probe used in these hybridizations was an oligodeoxyribonucleotide of sequence 5'TAATGGTCTCGTGCGAATTCTTGAT3' (SEQ ID NO:4) anticomplementary to the derivatized oligonucleotide. A signal observed after autoradiography, indicated that the derivatized oligonucleotide had been truly joined to the mRNA.

The remaining nine tenth of the mRNAs which had reacted with the derivatized oligonucleotide was reverse transcribed as follows. A reverse transcription reaction was

carried out with reverse transcriptase following the manufacturer's instructions and 50 pmol of nonamers with random sequence as primers.

To ensure that reverse transcription had been carried out through the cap structure, two types of experiments were performed.

5 In the first approach, after elimination of RNA of the cDNA:RNA heteroduplexes obtained from the reverse transcription reaction by an alkaline hydrolysis, a portion of the resulting single stranded cDNAs was spotted on a positively charged membrane and hybridized, using conventional methods, to a ³²P labeled probe having a sequence identical to that of the derivatized oligonucleotide. Control spots containing, 1 pmol, 100 fmol, 50 fmol,
10 10 fmol and 1 fmol of a control oligodeoxyribonucleotide of sequence identical to that of the derivatized oligonucleotide were included. The signal observed in the spots containing the cDNA indicated that approximately 15 fmol of the derivatized oligonucleotide had been reverse transcribed. These results demonstrate that the reverse transcription can be performed through the cap and, in particular, that reverse transcriptase crosses the 5'-P-P-P-
15 5' bond of the cap of eukaryotic messenger RNAs.

In the second type of experiment, the single stranded cDNAs obtained from the above first strand synthesis were used as template for PCR reactions. Two types of reactions were carried out. First, specific amplification of the mRNAs for alpha globin, dehydrogenase, pp15 and elongation factor E4 were carried out using the following pairs of
20 oligodeoxyribonucleotide primers.

alpha-globin

GLO-S: 5'CCG ACA AGA CCA ACG TCA AGG CCG C3' (SEQ ID NO:5)

GLO-As: 5'TCA CCA GCA GGC AGT GGC TTA GGA G 3' (SEQ ID NO:6)

25

dehydrogenase

3 DH-S: 5'AGT GAT TCC TGC TAC TTT GGA TGG C3' (SEQ ID NO:7)

3 DH-As: 5'GCT TGG TCT TGT TCT GGA GTT TAG A3' (SEQ ID NO:8)

30

pp15

PP15-S: 5'TCC AGA ATG GGA GAC AAG CCA ATT T3' (SEQ ID NO:9)

PP15-As: 5' AGG GAG GAG GAA ACA GCG TGA GTC C3' (SEQ ID NO:10)

Elongation factor E4

EFA1-S: 5' ATG GGA AAG GAA AAG ACT CAT ATC A3' (SEQ ID NO:11)

5 EF1A-As: 5' AGC AGC AAC AAT CAG GAC AGC ACA G3' (SEQ ID NO:12)

Second, non specific amplifications were also carried out with the antisense oligodeoxyribonucleotides of the pairs described above and with a primer derived from the sequence of the derivatized oligodeoxyribonucleotide
10 (5' ATCAAGAATTCGCACGAGACCATTAA3') (SEQ ID NO:13).

One twentieth of the following RT-PCR product samples were run on a 1.5% agarose gel and stained with ethidium bromide.

Sample 1: The products of a PCR reaction using the globin primers of SEQ ID NOs 5 and 6 in the presence of cDNA.

15 Sample 2: The products of a PCR reaction using the globin primers of SEQ ID NOs 5 and 6 in the absence of added cDNA.

Sample 3: The products of a PCR reaction using the dehydrogenase primers of SEQ ID NOs 7 and 8 in the presence of cDNA.

20 Sample 4: The products of a PCR reaction using the dehydrogenase primers of SEQ ID NOs 7 and 8 in the absence of added cDNA.

Sample 5: The products of a PCR reaction using the pp15 primers of SEQ ID NOs 9 and 10 in the presence of cDNA.

Sample 6: The products of a PCR reaction using the pp15 primers of SEQ ID NOs 9 and 10 in the absence of added cDNA.

25 Sample 7: The products of a PCR reaction using the EIF4 primers of SEQ ID NOs 11 and 12 in the presence of added cDNA.

Sample 8: The products of a PCR reaction using the EIF4 primers of SEQ ID NOs 11 and 12 in the absence of added cDNA.

30 A band of the size expected for the PCR product was observed only in samples 1, 3, 5 and 7, thus indicating the presence of the corresponding sequence in the cDNA population.

PCR reactions were also carried out with the antisense oligonucleotides of the globin and dehydrogenase primers (SEQ ID NOs 6 and 8) and an oligonucleotide whose sequence corresponds to that of the derivatized oligonucleotide. The presence of PCR products of the expected size in the samples equivalent to above samples 1 and 3 indicated that the derivatized oligonucleotide had been linked to mRNA.

The above examples summarize the chemical procedure for enriching mRNAs for those having intact 5' ends as illustrated in Figure 1. Further detail regarding the chemical approaches for obtaining such mRNAs are disclosed in International Application No. WO96/34981, published November 7, 1996, which is incorporated herein by reference. Strategies based on the above chemical modifications to the 5' cap structure may be utilized to generate cDNAs selected to include the 5' ends of the mRNAs from which they derived. In one version of such procedures, the 5' ends of the mRNAs are modified as described above. Thereafter, a reverse transcription reaction is conducted to extend a primer complementary to the 5' end of the mRNA. Single stranded RNAs are eliminated to obtain a population of cDNA/mRNA heteroduplexes in which the mRNA includes an intact 5' end. The resulting heteroduplexes may be captured on a solid phase coated with a molecule capable of interacting with the molecule used to derivatize the 5' end of the mRNA. Thereafter, the strands of the heteroduplexes are separated to recover single stranded first cDNA strands which include the 5' end of the mRNA. Second strand cDNA synthesis may then proceed using conventional techniques. For example, the procedures disclosed in WO 96/34981 or in Carninci. *et al.*, *Genomics* 37:327-336, 1996, the disclosures of which are incorporated herein by reference, may be employed to select cDNAs which include the sequence derived from the 5' end of the coding sequence of the mRNA.

Following ligation of the oligonucleotide tag to the 5' cap of the mRNA, a reverse transcription reaction is conducted to extend a primer complementary to the mRNA to the 5' end of the mRNA. Following elimination of the RNA component of the resulting heteroduplex using standard techniques, second strand cDNA synthesis is conducted with a primer complementary to the oligonucleotide tag.

2. Enzymatic Methods for Obtaining mRNAs having Intact 5' Ends

Other techniques for selecting cDNAs extending to the 5' end of the mRNA from which they are derived are fully enzymatic. Some versions of these techniques are disclosed in Dumas Milne Edwards J.B. (Doctoral Thesis of Paris VI University, Le clonage des ADNc
5 complets: difficultes et perspectives nouvelles. Apports pour l'etude de la regulation de l'expression de la tryptophane hydroxylase de rat, 20 Dec. 1993), EP0 625572 and Kato *et al.*, *Gene* 150:243-250, 1994, the disclosures of which are incorporated herein by reference.

Briefly, in such approaches, isolated mRNA is treated with alkaline phosphatase to remove the phosphate groups present on the 5' ends of uncapped incomplete mRNAs.
10 Following this procedure, the cap present on full length mRNAs is enzymatically removed with a decapping enzyme such as T4 polynucleotide kinase or tobacco acid pyrophosphatase. An oligonucleotide, which may be either a DNA oligonucleotide or a DNA-RNA hybrid oligonucleotide having RNA at its 3' end, is then ligated to the phosphate present at the 5' end of the decapped mRNA using T4 RNA ligase. The oligonucleotide may include a
15 restriction site to facilitate cloning of the cDNAs following their synthesis. Example 12 below describes one enzymatic method based on the doctoral thesis of Dumas.

EXAMPLE 12

Enzymatic Approach for Obtaining 5' ESTs

20 Twenty micrograms of PolyA+ RNA were dephosphorylated using Calf Intestinal Phosphatase (Biolabs). After a phenol chloroform extraction, the cap structure of mRNA was hydrolysed using the Tobacco Acid Pyrophosphatase (purified as described by Shinshi *et al.*, *Biochemistry* 15: 2185-2190, 1976) and a hemi 5'DNA/RNA-3' oligonucleotide having an unphosphorylated 5' end, a stretch of adenosine ribophosphate at the 3' end, and an EcoRI
25 site near the 5' end was ligated to the 5'P ends of mRNA using the T4 RNA ligase (Biolabs). Oligonucleotides suitable for use in this procedure are preferably 30 to 50 bases in length. Oligonucleotides having an unphosphorylated 5' end may be synthesized by adding a fluorochrome at the 5' end. The inclusion of a stretch of adenosine ribophosphates at the 3' end of the oligonucleotide increases ligation efficiency. It will be appreciated that the
30 oligonucleotide may contain cloning sites other than EcoRI.

Following ligation of the oligonucleotide to the phosphate present at the 5' end of the decapped mRNA, first and second strand cDNA synthesis is carried out using conventional methods or those specified in EP0 625,572 and Kato *et al. supra*, and Dumas Milne Edwards, *supra*, the disclosures of which are incorporated herein by reference. The resulting cDNA may then be ligated into vectors such as those disclosed in Kato *et al. supra* or other nucleic acid vectors known to those skilled in the art using techniques such as those described in Sambrook *et al.*, Molecular Cloning: A Laboratory Manual 2d Ed., Cold Spring Harbor Laboratory Press, 1989, the disclosure of which is incorporated herein by reference.

10

II. Obtention and Characterization of the 5' ESTs of the Present Invention

The 5' ESTs of the present invention were obtained using the aforementioned chemical and enzymatic approaches for enriching mRNAs for those having intact 5' ends as described below.

15

1. Obtention of 5' ESTS Using mRNAs with Intact 5' Ends

First, mRNAs were prepared as described in Example 13 below.

EXAMPLE 13

20

Preparation of mRNA With Intact 5' Ends

Total human RNAs or polyA⁺ RNAs derived from 29 different tissues were respectively purchased from LABIMO and CLONTECH and used to generate 44 cDNA libraries as follows. The purchased RNA had been isolated from cells or tissues using acid guanidium thiocyanate-phenol-chloroform extraction (Chomczynski and Sacchi, *Analytical Biochemistry* 162:156-159, 1987). PolyA⁺ RNA was isolated from total RNA (LABIMO) by two passes of oligo dT chromatography, as described by Aviv and Leder, *Proc. Natl. Acad. Sci. USA* 69:1408-1412, 1972 in order to eliminate ribosomal RNA.

25

The quality and the integrity of the polyA⁺ RNAs were checked. Northern blots hybridized with a globin probe were used to confirm that the mRNAs were not degraded. Contamination of the polyA⁺ mRNAs by ribosomal sequences was checked using Northern blots and a probe derived from the sequence of the 28S rRNA. Preparations of mRNAs with

30

less than 5% of rRNAs were used in library construction. To avoid constructing libraries with RNAs contaminated by exogenous sequences (prokaryotic or fungal), the presence of bacterial 16S ribosomal sequences or of two highly expressed fungal mRNAs was examined using PCR.

5 Following preparation of the mRNAs, the above described chemical and/or the enzymatic procedures for enriching mRNAs for those having intact 5' ends were employed to obtain 5' ESTs from various tissues. In both approaches, an oligonucleotide tag was attached to the 5' ends of the mRNAs. The oligonucleotide tag had an EcoRI site therein to facilitate later cloning procedures. To facilitate the processing of single stranded and double
10 stranded cDNA obtained in the construction of the libraries, the same nucleotidic sequence was used to design the ligated oligonucleotide in both chemical and enzymatic approaches. Nevertheless, in the chemical procedure, the tag used was an oligodeoxyribonucleotide which was linked to the cap of the mRNA whereas in the enzymatic ligation, the tag was a chimeric hemi 5'DNA/RNA3' oligonucleotide which was ligated to the 5' end of decapped mRNA as
15 described in example 12.

Following attachment of the oligonucleotide tag to the mRNA by either the chemical or enzymatic methods, the integrity of the mRNA was examined by performing a Northern blot with 200 to 500 ng of mRNA using a probe complementary to the oligonucleotide tag before performing the first strand synthesis as described in example 14.

20

EXAMPLE 14

cDNA Synthesis Using mRNA Templates Having Intact 5' Ends

For the mRNAs joined to oligonucleotide tags using both the chemical and enzymatic methods, first strand cDNA synthesis was performed using the Superscript II (Gibco BRL) or
25 the Rnase H Minus M-MLV (Promega) reverse transcriptase with random nonamers as primers. In order to protect internal EcoRI sites in the cDNA from digestion at later steps in the procedure, methylated dCTP was used for first strand synthesis. After removal of RNA by an alkaline hydrolysis, the first strand of cDNA was precipitated using isopropanol in order to eliminate residual primers.

30 For both the chemical and the enzymatic methods, the second strand of the cDNA was synthesized with a Klenow fragment using a primer corresponding to the 5' end of the

ligated oligonucleotide described in Example 12. Preferably, the primer is 20-25 bases in length. Methylated dCTP was also used for second strand synthesis in order to protect internal EcoRI sites in the cDNA from digestion during the cloning process.

Following cDNA synthesis, the cDNAs were cloned into pBlueScript as described in
5 Example 15 below.

EXAMPLE 15

Cloning of cDNAs derived from mRNA with intact 5' ends into BlueScript

Following second strand synthesis, the ends of the cDNA were blunted with T4 DNA
10 polymerase (Biolabs) and the cDNA was digested with EcoRI. Since methylated dCTP was used during cDNA synthesis, the EcoRI site present in the tag was the only hemi-methylated site, hence the only site susceptible to EcoRI digestion. The cDNA was then size fractionated using exclusion chromatography (AcA, Biosepra) and fractions corresponding to cDNAs of more than 150 bp were pooled and ethanol precipitated. The cDNA was directionally cloned
15 into the SmaI and EcoRI ends of the phagemid pBlueScript vector (Stratagene). The ligation mixture was electroporated into bacteria and propagated under appropriate antibiotic selection.

Clones containing the oligonucleotide tag attached were then selected as described in
Example 16 below.

20

EXAMPLE 16

Selection of Clones Having the Oligonucleotide Tag Attached Thereto

The plasmid DNAs containing 5' EST libraries made as described above were purified (Qiagen). A positive selection of the tagged clones was performed as follows.
25 Briefly, in this selection procedure, the plasmid DNA was converted to single stranded DNA using gene II endonuclease of the phage F1 in combination with an exonuclease (Chang *et al.*, *Gene* 127:95-8, 1993) such as exonuclease III or T7 gene 6 exonuclease. The resulting single stranded DNA was then purified using paramagnetic beads as described by Fry *et al.*, *Biotechniques*, 13: 124-131, 1992. In this procedure, the single stranded DNA was
30 hybridized with a biotinylated oligonucleotide having a sequence corresponding to the 3' end of the oligonucleotide described in Example 13. Preferably, the primer has a length of 20-25

bases. Clones including a sequence complementary to the biotinylated oligonucleotide were captured by incubation with streptavidin coated magnetic beads followed by magnetic selection. After capture of the positive clones, the plasmid DNA was released from the magnetic beads and converted into double stranded DNA using a DNA polymerase such as the ThermoSequenase obtained from Amersham Pharmacia Biotech. Alternatively, protocols such as the one described in the Gene Trapper kit available from Gibco BRL may be used. The double stranded DNA was then electroporated into bacteria. The percentage of positive clones having the 5' tag oligonucleotide was estimated to typically rank between 90 and 98% using dot blot analysis.

Following electroporation, the libraries were ordered in 384-microtiter plates (MTP). A copy of the MTP was stored for future needs. Then the libraries were transferred into 96 MTP and sequenced as described below.

EXAMPLE 17

Sequencing of Inserts in Selected Clones

Plasmid inserts were first amplified by PCR on PE 9600 thermocyclers (Perkin-Elmer, Applied Biosystems Division, Foster City, CA), using standard SETA-A and SETA-B primers (Genset SA), AmpliTaqGold (Perkin-Elmer), dNTPs (Boehringer), buffer and cycling conditions as recommended by the Perkin-Elmer Corporation.

PCR products were then sequenced using automatic ABI Prism 377 sequencers (Perkin Elmer). Sequencing reactions were performed using PE 9600 thermocyclers with standard dye-primer chemistry and ThermoSequenase (Amersham Pharmacia Biotech). The primers used were either T7 or 21M13 (available from Genset SA) as appropriate. The primers were labeled with the JOE, FAM, ROX and TAMRA dyes. The dNTPs and ddNTPs used in the sequencing reactions were purchased from Boehringer. Sequencing buffer, reagent concentrations and cycling conditions were as recommended by Amersham.

Following the sequencing reaction, the samples were precipitated with ethanol, resuspended in formamide loading buffer, and loaded on a standard 4% acrylamide gel. Electrophoresis was performed for 2.5 hours at 3000V on an ABI 377 sequencer, and the sequence data were collected and analyzed using the ABI Prism DNA Sequencing Analysis Software, version 2.1.2.

2. Computer analysis of the Obtained 5' ESTs: Construction of NetGene and SignalTag databases

The sequence data from the 44 cDNA libraries made as described above were transferred to a proprietary database, where quality control and validation steps were performed. A proprietary base-caller, working using a Unix system, automatically flagged suspect peaks, taking into account the shape of the peaks, the inter-peak resolution, and the noise level. The proprietary base-caller also performed an automatic trimming. Any stretch of 25 or fewer bases having more than 4 suspect peaks was considered unreliable and was discarded. Sequences corresponding to cloning vector or ligation oligonucleotides were automatically removed from the EST sequences. However, the resulting EST sequences may contain 1 to 5 bases belonging to the above mentioned sequences at their 5' end. If needed, these can easily be removed on a case to case basis.

Following sequencing as described above, the sequences of the 5' ESTs were entered in NetGene™, a proprietary database called for storage and manipulation as described below. It will be appreciated by those skilled in the art that the data could be stored and manipulated on any medium which can be read and accessed by a computer. Computer readable media include magnetically, optically, or electronically readable media. For example, the computer readable media may be a hard disc, a floppy disc, a magnetic tape, CD-ROM, RAM, or ROM as well as other types of other media known to those skilled in the art.

In addition, the sequence data may be stored and manipulated in a variety of data processor programs in a diversity of formats. For instance, the sequence data may be stored as text in a word processing file, such as Microsoft WORD or WORDPERFECT or as an ASCII file in a variety of database programs familiar to those of skill in the art, such as DB2, SYBASE, or ORACLE.

The computer readable media on which the sequence information is stored may be in a personal computer, a network, a server or other computer systems known to those skilled in the art. The computer or other system preferably includes the storage media described above, and a processor for accessing and manipulating the sequence data. Once the sequence data has been stored, it may be manipulated and searched to locate those stored sequences which contain a desired nucleic acid sequence or which encode a protein having a particular functional domain. For example, the stored sequence information may be compared to other

known sequences to identify homologies, motifs implicated in biological function, or structural motifs.

Programs which may be used to search or compare the stored sequences include the MacPattern (EMBL), BLAST, and BLAST2 program series (NCBI), basic local alignment
5 search tool programs for nucleotide (BLASTN) and peptide (BLASTX) comparisons (Altschul *et al*, *J. Mol. Biol.* **215**: 403, 1990) and FASTA (Pearson and Lipman, *Proc. Natl. Acad. Sci. USA* **85**: 2444, 1988). The BLAST programs then extend the alignments on the basis of defined match and mismatch criteria.

Motifs which may be detected using the above programs and those described in
10 Example 28 include sequences encoding leucine zippers, helix-turn-helix motifs, glycosylation sites, ubiquitination sites, alpha helices, and beta sheets, signal sequences encoding signal peptides which direct the secretion of the encoded proteins, sequences implicated in transcription regulation such as homeoboxes, acidic stretches, enzymatic active sites, substrate binding sites, and enzymatic cleavage sites.

15 Before searching the cDNAs in the NetGene™ database for sequence motifs of interest, cDNAs derived from mRNAs which were not of interest were identified and eliminated from further consideration as described in Example 18 below.

EXAMPLE 18

20 Elimination of Undesired Sequences from Further Consideration

5' ESTs in the NetGene™ database which were derived from undesired sequences such as transfer RNAs, ribosomal RNAs, mitochondrial RNAs, prokaryotic RNAs, fungal RNAs, Alu sequences, L1 sequences, or repeat sequences were identified using the FASTA and BLASTN programs with the parameters listed in Table I.

25 To eliminate 5' ESTs encoding tRNAs from further consideration, the 5' EST sequences were compared to the sequences of 1190 known tRNAs obtained from EMBL release 38, of which 100 were human. The comparison was performed using FASTA on both strands of the 5' ESTs. Sequences having more than 80% homology over more than 60 nucleotides were identified as tRNA. Of the 144,341 sequences screened, 26 were identified
30 as tRNAs and eliminated from further consideration.

To eliminate 5' ESTs encoding rRNAs from further consideration, the 5' EST sequences were compared to the sequences of 2497 known rRNAs obtained from EMBL release 38, of which 73 were human. The comparison was performed using BLASTN on both strands of the 5' ESTs with the parameter S=108. Sequences having more than 80%
5 homology over stretches longer than 40 nucleotides were identified as rRNAs. Of the 144,341 sequences screened, 3,312 were identified as rRNAs and eliminated from further consideration.

To eliminate 5' ESTs encoding mtRNAs from further consideration, the 5' EST sequences were compared to the sequences of the two known mitochondrial genomes for
10 which the entire genomic sequences are available and all sequences transcribed from these mitochondrial genomes including tRNAs, rRNAs, and mRNAs for a total of 38 sequences. The comparison was performed using BLASTN on both strands of the 5' ESTs with the parameter S=108. Sequences having more than 80% homology over stretches longer than 40 nucleotides were identified as mtRNAs. Of the 144,341 sequences screened, 6,110 were
15 identified as mtRNAs and eliminated from further consideration.

Sequences which might have resulted from exogenous contaminants were eliminated from further consideration by comparing the 5' EST sequences to release 46 of the EMBL bacterial and fungal divisions using BLASTN with the parameter S=144. All sequences having more than 90% homology over at least 40 nucleotides were identified as exogenous
20 contaminants. Of the 42 cDNA libraries examined, the average percentages of prokaryotic and fungal sequences contained therein were 0.2% and 0.5% respectively. Among these sequences, only one could be identified as a sequence specific to fungi. The others were either fungal or prokaryotic sequences having homologies with vertebrate sequences or including repeat sequences which had not been masked during the electronic comparison.

25 In addition, the 5' ESTs were compared to 6093 Alu sequences and 1115 L1 sequences to mask 5' ESTs containing such repeat sequences. 5' ESTs including THE and MER repeats, SSTR sequences or satellite, micro-satellite, or telomeric repeats were also eliminated from further consideration. On average, 11.5% of the sequences in the libraries contained repeat sequences. Of this 11.5%, 7% contained Alu repeats, 3.3% contained L1
30 repeats and the remaining 1.2% were derived from the other screened types of repetitive sequences. These percentages are consistent with those found in cDNA libraries prepared by

other groups. For example, the cDNA libraries of Adams *et al.* contained between 0% and 7.4% Alu repeats depending on the source of the RNA which was used to prepare the cDNA library (Adams *et al.*, *Nature* 377:174, 1996).

- 5 The sequences of those 5' ESTs remaining after the elimination of undesirable sequences were compared with the sequences of known human mRNAs to determine the accuracy of the sequencing procedures described above.

EXAMPLE 19

10 Measurement of Sequencing Accuracy by Comparison to Known Sequences

To further determine the accuracy of the sequencing procedure described above, the sequences of 5' ESTs derived from known sequences were identified and compared to the original known sequences. First, a FASTA analysis with overhangs shorter than 5 bp on both ends was conducted on the 5' ESTs to identify those matching an entry in the public human mRNA database. The 6655 5' ESTs which matched a known human mRNA were then
15 realigned with their cognate mRNA and dynamic programming was used to include substitutions, insertions, and deletions in the list of "errors" which would be recognized. Errors occurring in the last 10 bases of the 5' EST sequences were ignored to avoid the inclusion of spurious cloning sites in the analysis of sequencing accuracy.

- 20 This analysis revealed that the sequences incorporated in the NetGene™ database had an accuracy of more than 99.5%.

To determine the efficiency with which the above selection procedures select cDNAs which include the 5' ends of their corresponding mRNAs, the following analysis was
25 performed.

EXAMPLE 20

Determination of Efficiency of 5' EST Selection

- To determine the efficiency at which the above selection procedures isolated 5' ESTs
30 which included sequences close to the 5' end of the mRNAs from which they derived, the sequences of the ends of the 5' ESTs derived from the elongation factor 1 subunit α and

ferritin heavy chain genes were compared to the known cDNA sequences of these genes. Since the transcription start sites of both genes are well characterized, they may be used to determine the percentage of derived 5' ESTs which included the authentic transcription start sites.

5 For both genes, more than 95% of the obtained 5' ESTs actually included sequences close to or upstream of the 5' end of the corresponding mRNAs.

To extend the analysis of the reliability of the procedures for isolating 5' ESTs from ESTs in the NetGene™ database, a similar analysis was conducted using a database composed of human mRNA sequences extracted from GenBank database release 97 for
10 comparison. The 5' ends of more than 85% of 5' ESTs derived from mRNAs included in the GeneBank database were located close to the 5' ends of the known sequence. As some of the mRNA sequences available in the GenBank database are deduced from genomic sequences, a 5' end matching with these sequences will be counted as an internal match. Thus, the method used here underestimates the yield of ESTs including the authentic 5' ends
15 of their corresponding mRNAs.

The EST libraries made above included multiple 5' ESTs derived from the same mRNA. The sequences of such 5' ESTs were compared to one another and the longest 5' ESTs for each mRNA were identified. Overlapping cDNAs were assembled into continuous
20 sequences (contigs). The resulting continuous sequences were then compared to public databases to gauge their similarity to known sequences, as described in Example 21 below.

EXAMPLE 21

Clustering of the 5' ESTs and Calculation of Novelty Indices for cDNA Libraries

25 For each sequenced EST library, the sequences were clustered by the 5' end. Each sequence in the library was compared to the others with BLASTN2 (direct strand, parameters S=107). ESTs with High Scoring Segment Pairs (HSPs) at least 25 bp long, having 95% identical bases and beginning closer than 10 bp from each EST 5' end were grouped. The longest sequence found in the cluster was used as representative of the group. A global
30 clustering between libraries was then performed leading to the definition of super-contigs.

To assess the yield of new sequences within the EST libraries, a novelty rate (NR) was defined as: $NR = 100 \times (\text{Number of new unique sequences found in the library} / \text{Total number of sequences from the library})$. Typically, novelty rating ranged between 10% and 41% depending on the tissue from which the EST library was obtained. For most of the
5 libraries, the random sequencing of 5' EST libraries was pursued until the novelty rate reached 20%.

Following characterization as described above, the collection of 5' ESTs in NetGene™ was screened to identify those 5' ESTs bearing potential signal sequences as
10 described in Example 22 below.

EXAMPLE 22

Identification of Potential Signal Sequences in 5' ESTs

The 5' ESTs in the NetGene™ database were screened to identify those having an
15 uninterrupted open reading frame (ORF) longer than 45 nucleotides beginning with an ATG codon and extending to the end of the EST. Approximately half of the cDNA sequences in NetGene™ contained such an ORF. The ORFs of these 5' ESTs were then searched to identify potential signal motifs using slight modifications of the procedures disclosed in Von Heijne, *Nucleic Acids Res.* 14:4683-4690, 1986, the disclosure of which is incorporated
20 herein by reference. Those 5' EST sequences encoding a stretch of at least 15 amino acid long with a score of at least 3.5 in the Von Heijne signal peptide identification matrix were considered to possess a signal sequence. Those 5' ESTs which matched a known human mRNA or EST sequence and had a 5' end more than 20 nucleotides downstream of the known 5' end were excluded from further analysis. The remaining cDNAs having signal
25 sequences therein were included in a database called SignalTag™.

To confirm the accuracy of the above method for identifying signal sequences, the analysis of Example 23 was performed.

EXAMPLE 23Confirmation of Accuracy of Identification of Potential Signal Sequences in 5' ESTs

The accuracy of the above procedure for identifying signal sequences encoding signal peptides was evaluated by applying the method to the 43 amino acids located at the N terminus of all human SwissProt proteins. The computed Von Heijne score for each protein was compared with the known characterization of the protein as being a secreted protein or a non-secreted protein. In this manner, the number of non-secreted proteins having a score higher than 3.5 (false positives) and the number of secreted proteins having a score lower than 3.5 (false negatives) could be calculated.

Using the results of the above analysis, the probability that a peptide encoded by the 5' region of the mRNA is in fact a genuine signal peptide based on its Von Heijne's score was calculated based on either the assumption that 10% of human proteins are secreted or the assumption that 20% of human proteins are secreted. The results of this analysis are shown in Figure 2 and table IV.

Using the above method of identification of secretory proteins, 5' ESTs of the following polypeptides known to be secreted were obtained: human glucagon, gamma interferon induced monokine precursor, secreted cyclophilin-like protein, human pleiotropin, and human biotinidase precursor. Thus, the above method successfully identified those 5' ESTs which encode a signal peptide.

To confirm that the signal peptide encoded by the 5' ESTs actually functions as a signal peptide, the signal sequences from the 5' ESTs may be cloned into a vector designed for the identification of signal peptides. Such vectors are designed to confer the ability to grow in selective medium only to host cells containing a vector with an operably linked signal sequence. For example, to confirm that a 5' EST encodes a genuine signal peptide, the signal sequence of the 5' EST may be inserted upstream and in frame with a non-secreted form of the yeast invertase gene in signal peptide selection vectors such as those described in U.S. Patent No. 5,536,637, the disclosure of which is incorporated herein by reference. Growth of host cells containing signal sequence selection vectors with the correctly inserted 5' EST signal sequence confirms that the 5' EST encodes a genuine signal peptide.

Alternatively, the presence of a signal peptide may be confirmed by cloning the extended cDNAs obtained using the ESTs into expression vectors such as pXT1 (as described below in example 30), or by constructing promoter-signal sequence-reporter gene vectors which encode fusion proteins between the signal peptide and an assayable reporter protein. After introduction of these vectors into a suitable host cell, such as COS cells or NIH 3T3 cells, the growth medium may be harvested and analyzed for the presence of the secreted protein. The medium from these cells is compared to the medium from control cells containing vectors lacking the signal sequence or extended cDNA insert to identify vectors which encode a functional signal peptide or an authentic secreted protein.

Those 5' ESTs which encoded a signal peptide, as determined by the method of Example 22 above, were further grouped into four categories based on their homology to known sequences as described in Example 24 below.

EXAMPLE 24

Categorization of 5' ESTs Encoding a Signal Peptide

Those 5' ESTs having a sequence not matching any known vertebrate sequence nor any publicly available EST sequence were designated "new." Of the sequences in the SignalTag™ database, 947 of the 5' ESTs having a Von Heijne's score of at least 3.5 fell into this category.

Those 5' ESTs having a sequence not matching any vertebrate sequence but matching a publicly known EST were designated "EST-ext", provided that the known EST sequence was extended by at least 40 nucleotides in the 5' direction. Of the sequences in the SignalTag™ database, 150 of the 5' ESTs having a Von Heijne's score of at least 3.5 fell into this category.

Those ESTs not matching any vertebrate sequence but matching a publicly known EST without extending the known EST by at least 40 nucleotides in the 5' direction were designated "EST." Of the sequences in the SignalTag™ database, 599 of the 5' ESTs having a Von Heijne's score of at least 3.5 fell into this category.

Those 5' ESTs matching a human mRNA sequence but extending the known sequence by at least 40 nucleotides in the 5' direction were designated "VERT-ext." Of the sequences in the SignalTag™ database, 23 of the 5' ESTs having a Von Heijne's score of at

least 3.5 fell into this category. Included in this category was a 5' EST which extended the known sequence of the human translocase mRNA by more than 200 bases in the 5' direction. A 5' EST which extended the sequence of a human tumor suppressor gene in the 5' direction was also identified.

5 Table V shows the distribution of 5' ESTs in each category and the number of 5' ESTs in each category having a given minimum von Heijne's score.

3. Evaluation of Spatial and Temporal Expression of mRNAs Corresponding to the 5'ESTs or Extended cDNAs

10

Each of the 5' ESTs was also categorized based on the tissue from which its corresponding mRNA was obtained, as described below in Example 25.

EXAMPLE 25

15

Categorization of Expression Patterns

Table VI shows the distribution of 5' ESTs in each of the above defined category with respect to the tissue from which the 5'ESTs of the corresponding mRNA were obtained.

20 Table II provides the sequence identification numbers of 5' EST sequences derived from muscle and other mesodermal tissues, the categories in which these sequences fall, and the von Heijne's score of the signal peptides which they encode. The 5' EST sequences and the amino acid sequences they encode are provided in the appended sequence listings. Table III provides the sequence ID numbers of the 5' ESTs and the sequences of the signal peptides which they encode. The sequences of the 5' ESTs and the polypeptides they encode are provided in the sequence listing appended hereto.

25

The sequences of DNA SEQ ID NOs: 38-305 can readily be screened for any errors therein and any sequence ambiguities can be resolved by resequencing a fragment containing such errors or ambiguities on both strands. Such fragments may be obtained from the plasmids stored in the inventors' laboratory or can be isolated using the techniques described herein. Resolution of any such ambiguities or errors may be facilitated by using primers
30 which hybridize to sequences located close to the ambiguous or erroneous sequences. For example, the primers may hybridize to sequences within 50-75 bases of the ambiguity or

error. Upon resolution of an error or ambiguity, the corresponding corrections can be made in the protein sequences encoded by the DNA containing the error or ambiguity.

In addition to categorizing the 5' ESTs with respect to their tissue of origin, the spatial and temporal expression patterns of the mRNAs corresponding to the 5' ESTs, as well as their expression levels, may be determined as described in Example 26 below. Characterization of the spatial and temporal expression patterns and expression levels of these mRNAs is useful for constructing expression vectors capable of producing a desired level of gene product in a desired spatial or temporal manner, as will be discussed in more detail below.

Furthermore, 5' ESTs whose corresponding mRNAs are associated with disease states may also be identified. For example, a particular disease may result from the lack of expression, over expression, or under expression of an mRNA corresponding to a 5' EST. By comparing mRNA expression patterns and quantities in samples taken from healthy individuals with those from individuals suffering from a particular disease, 5' ESTs responsible for the disease may be identified.

It will be appreciated that the results of the above characterization procedures for 5' ESTs also apply to extended cDNAs (obtainable as described below) which contain sequences adjacent to the 5' ESTs. It will also be appreciated that if desired, characterization may be delayed until extended cDNAs have been obtained rather than characterizing the ESTs themselves.

EXAMPLE 26

Evaluation of Expression Levels and Patterns of mRNAs

Corresponding to 5' ESTs or Extended cDNAs

Expression levels and patterns of mRNAs corresponding to 5' ESTs or extended cDNAs (obtainable as described below in example 27) may be analyzed by solution hybridization with long probes as described in International Patent Application No. WO 97/05277, the entire contents of which are hereby incorporated by reference. Briefly, a 5' EST, extended cDNA, or fragment thereof corresponding to the gene encoding the mRNA to be characterized is inserted at a cloning site immediately downstream of a bacteriophage (T3,

T7 or SP6) RNA polymerase promoter to produce antisense RNA. Preferably, the 5' EST or extended cDNA has 100 or more nucleotides. The plasmid is linearized and transcribed in the presence of ribonucleotides comprising modified ribonucleotides (*i.e.* biotin-UTP and DIG-UTP). An excess of this doubly labeled RNA is hybridized in solution with mRNA isolated
5 from cells or tissues of interest. The hybridizations are performed under standard stringent conditions (40-50°C for 16 hours in an 80% formamide, 0.4 M NaCl buffer, pH 7-8). The unhybridized probe is removed by digestion with ribonucleases specific for single-stranded RNA (*i.e.* RNases CL3, T1, Phy M, U2 or A). The presence of the biotin-UTP modification enables capture of the hybrid on a microtitration plate coated with streptavidin. The presence
10 of the DIG modification enables the hybrid to be detected and quantified by ELISA using an anti-DIG antibody coupled to alkaline phosphatase.

The 5' ESTs, extended cDNAs, or fragments thereof may also be tagged with nucleotide sequences for the serial analysis of gene expression (SAGE) as disclosed in UK Patent Application No. 2 305 241 A, the entire contents of which are incorporated by
15 reference. In this method, cDNAs are prepared from a cell, tissue, organism or other source of nucleic acid for which gene expression patterns must be determined. The resulting cDNAs are separated into two pools. The cDNAs in each pool are cleaved with a first restriction endonuclease, called an anchoring enzyme, having a recognition site which is likely to be present at least once in most cDNAs. The fragments which contain the 5' or 3' most region
20 of the cleaved cDNA are isolated by binding to a capture medium such as streptavidin coated beads. A first oligonucleotide linker having a first sequence for hybridization of an amplification primer and an internal restriction site for a so-called tagging endonuclease is ligated to the digested cDNAs in the first pool. Digestion with the second endonuclease produces short tag fragments from the cDNAs.

25 A second oligonucleotide having a second sequence for hybridization of an amplification primer and an internal restriction site is ligated to the digested cDNAs in the second pool. The cDNA fragments in the second pool are also digested with the tagging endonuclease to generate short tag fragments derived from the cDNAs in the second pool. The tags resulting from digestion of the first and second pools with the anchoring enzyme and
30 the tagging endonuclease are ligated to one another to produce so-called ditags. In some embodiments, the ditags are concatamerized to produce ligation products containing from 2

to 200 ditags. The tag sequences are then determined and compared to the sequences of the 5' ESTs or extended cDNAs to determine which 5' ESTs or extended cDNAs are expressed in the cell, tissue, organism, or other source of nucleic acids from which the tags were derived. In this way, the expression pattern of the 5' ESTs or extended cDNAs in the cell,
5 tissue, organism, or other source of nucleic acids is obtained.

Quantitative analysis of gene expression may also be performed using arrays. As used herein, the term array means a one dimensional, two dimensional, or multidimensional arrangement of full length cDNAs (*i.e.* extended cDNAs which include the coding sequence for the signal peptide, the coding sequence for the mature protein, and a stop codon),
10 extended cDNAs, 5' ESTs or fragments thereof of sufficient length to permit specific detection of gene expression. Preferably, the fragments are at least 15 nucleotides in length. More preferably, the fragments are at least 100 nucleotide long. More preferably, the fragments are more than 100 nucleotides in length. In some embodiments, the fragments may be more than 500 nucleotide long.

15 For example, quantitative analysis of gene expression may be performed with full length cDNAs as defined below, extended cDNAs, 5' ESTs, or fragments thereof in a complementary DNA microarray as described by Schena *et al.* (*Science* 270:467-470, 1995; *Proc. Natl. Acad. Sci. U.S.A.* 93:10614-10619, 1996). Full length cDNAs, extended cDNAs, 5' ESTs or fragments thereof are amplified by PCR and arrayed from 96-well microtiter
20 plates onto silylated microscope slides using high-speed robotics. Printed arrays are incubated in a humid chamber to allow rehydration of the array elements and rinsed, once in 0.2% SDS for 1 min, twice in water for 1 min and once for 5 min in sodium borohydride solution. The arrays are submerged in water for 2 min at 95°C, transferred into 0.2% SDS for 1 min, rinsed twice with water, air dried and stored in the dark at 25°C.

25 Cell or tissue mRNA is isolated or commercially obtained and probes are prepared by a single round of reverse transcription. Probes are hybridized to 1 cm² microarrays under a 14 x 14 mm glass coverslip for 6-12 hours at 60°C. Arrays are washed for 5 min at 25°C in low stringency wash buffer (1 x SSC/0.2% SDS), then for 10 min at room temperature in high stringency wash buffer (0.1 x SSC/0.2% SDS). Arrays are scanned in 0.1 x SSC using a
30 fluorescence laser scanning device fitted with a custom filter set. Accurate differential

expression measurements are obtained by taking the average of the ratios of two independent hybridizations.

Quantitative analysis of the expression of genes may also be performed with full length cDNAs, extended cDNAs, 5' ESTs, or fragments thereof in complementary DNA arrays as described by Pietu *et al.* (*Genome Research* 6:492-503, 1996). The full length cDNAs, extended cDNAs, 5' ESTs or fragments thereof are PCR amplified and spotted on membranes. Then, mRNAs originating from various tissues or cells are labeled with radioactive nucleotides. After hybridization and washing in controlled conditions, the hybridized mRNAs are detected by phospho-imaging or autoradiography. Duplicate experiments are performed and a quantitative analysis of differentially expressed mRNAs is then performed.

Alternatively, expression analysis of the 5' ESTs or extended cDNAs can be done through high density nucleotide arrays as described by Lockhart *et al.* (*Nature Biotechnology* 14: 1675-1680, 1996) and Sosnowsky *et al.* (*Proc. Natl. Acad. Sci.* 94:1119-1123, 1997). Oligonucleotides of 15-50 nucleotides corresponding to sequences of the 5' ESTs or extended cDNAs are synthesized directly on the chip (Lockhart *et al.*, *supra*) or synthesized and then addressed to the chip (Sosnowsky *et al.*, *supra*). Preferably, the oligonucleotides are about 20 nucleotides in length.

cDNA probes labeled with an appropriate compound, such as biotin, digoxigenin or fluorescent dye, are synthesized from the appropriate mRNA population and then randomly fragmented to an average size of 50 to 100 nucleotides. The said probes are then hybridized to the chip. After washing as described in Lockhart *et al.*, *supra* and application of different electric fields (Sonowsky *et al.*, *supra.*), the dyes or labeling compounds are detected and quantified. Duplicate hybridizations are performed. Comparative analysis of the intensity of the signal originating from cDNA probes on the same target oligonucleotide in different cDNA samples indicates a differential expression of the mRNA corresponding to the 5' EST or extended cDNA from which the oligonucleotide sequence has been designed.

III. Use of 5' ESTs to Clone Extended cDNAs and to Clone the Corresponding Genomic DNAs

Once 5' ESTs which include the 5' end of the corresponding mRNAs have been selected using the procedures described above, they can be utilized to isolate extended cDNAs which contain sequences adjacent to the 5' ESTs. The extended cDNAs may include the entire coding sequence of the protein encoded by the corresponding mRNA, including the authentic translation start site, the signal sequence, and the sequence encoding the mature protein remaining after cleavage of the signal peptide. Such extended cDNAs are referred to herein as "full length cDNAs." Alternatively, the extended cDNAs may include only the sequence encoding the mature protein remaining after cleavage of the signal peptide, or only the sequence encoding the signal peptide.

Example 27 below describes a general method for obtaining extended cDNAs using 5' ESTs. Example 28 below provides experimental results, using the method explained in example 27, describing several extended cDNAs including the entire coding sequence and authentic 5' end of the corresponding mRNA for several secreted proteins.

The methods of Examples 27, 28, and 29 can also be used to obtain extended cDNAs which encode less than the entire coding sequence of the secreted proteins encoded by the genes corresponding to the 5' ESTs. In some embodiments, the extended cDNAs isolated using these methods encode at least 10 amino acids of one of the proteins encoded by the sequences of SEQ ID NOs: 38-305. In further embodiments, the extended cDNAs encode at least 20 amino acids of the proteins encoded by the sequences of SEQ ID NOs: 38-305. In further embodiments, the extended cDNAs encode at least 30 amino amino acids of the sequences of SEQ ID NOs: 38-305. In a preferred embodiment, the extended cDNAs encode a full length protein sequence, which includes the protein coding sequences of SEQ ID NOs: 38-305.

EXAMPLE 27

General Method for Using 5' ESTs to Clone and Sequence cDNAs which Include the Entire Coding Region and the Authentic 5' End of the Corresponding mRNA

The following general method has been used to quickly and efficiently isolate extended cDNAs having the authentic 5' ends of their corresponding mRNAs as well as

the full protein coding sequence and including sequence adjacent to the sequences of the 5' ESTs used to obtain them. This method may be applied to obtain extended cDNAs for any 5' EST in the NetGene™ database, including those 5' ESTs encoding polypeptides belonging to secreted proteins. The method is summarized in figure 3.

5

1. Obtention of Extended cDNAs

a) First strand synthesis

The method takes advantage of the known 5' sequence of the mRNA. A reverse transcription reaction is conducted on purified mRNA with a poly 14dT primer containing a
10 49 nucleotide sequence at its 5' end allowing the addition of a known sequence at the end of the cDNA which corresponds to the 3' end of the mRNA. For example, the primer may have the following sequence: 5'-ATC GTT GAG ACT CGT ACC AGC AGA GTC ACG AGA GAG ACT ACA CGG TAC TGG TTT TTT TTT TTT TTVN -3' (SEQ ID NO:14). Those skilled in the art will appreciate that other sequences may also be added to the poly dT
15 sequence and used to prime the first strand synthesis. Using this primer and a reverse transcriptase such as the Superscript II (Gibco BRL) or Rnase H Minus M-MLV (Promega) enzyme, a reverse transcript anchored at the 3' polyA site of the RNAs is generated.

After removal of the mRNA hybridized to the first cDNA strand by alkaline hydrolysis, the products of the alkaline hydrolysis and the residual poly dT primer are
20 eliminated with an exclusion column such as an Aca34 (Biosepra) matrix as explained in Example 11.

b) Second strand synthesis

A pair of nested primers on each end is designed based on the known 5' sequence from the 5' EST and the known 3' end added by the poly dT primer used in the first strand
25 synthesis. Softwares used to design primers are either based on GC content and melting temperatures of oligonucleotides, such as OSP (Illier and Green, *PCR Meth. Appl.* 1:124-128, 1991), or based on the octamer frequency disparity method (Griffais *et al.*, *Nucleic Acids Res.* 19: 3887-3891, 1991) such as PC-Rare (<http://bioinformatics.weizmann.ac.il/software/PC-Rare/doc/manuel.html>).

Preferably, the nested primers at the 5' end are separated from one another by four to nine bases. The 5' primer sequences may be selected to have melting temperatures and specificities suitable for use in PCR.

5 Preferably, the nested primers at the 3' end are separated from one another by four to nine bases. For example, the nested 3' primers may have the following sequences: (5'- CCA GCA GAG TCA CGA GAG AGA CTA CAC GG -3'(SEQ ID NO:15), and 5'- CAC GAG AGA GAC TAC ACG GTA CTG G -3' (SEQ ID NO:16). These primers were selected because they have melting temperatures and specificities compatible with their use in PCR. However, those skilled in the art will appreciate that other sequences may also be used as
10 primers.

The first PCR run of 25 cycles is performed using the Advantage Tth Polymerase Mix (Clontech) and the outer primer from each of the nested pairs. A second 20 cycle PCR using the same enzyme and the inner primer from each of the nested pairs is then performed on 1/2500 of the first PCR product. Thereafter, the primers and nucleotides
15 are removed.

2. Sequencing of Full Length Extended cDNAs or Fragments Thereof

Due to the lack of position constraints on the design of 5' nested primers compatible for PCR use using the OSP software, amplicons of two types are obtained.
20 Preferably, the second 5' primer is located upstream of the translation initiation codon thus yielding a nested PCR product containing the whole coding sequence. Such a full length extended cDNA undergoes a direct cloning procedure as described in section a. However, in some cases, the second 5' primer is located downstream of the translation initiation codon, thereby yielding a PCR product containing only part of the ORF. Such
25 incomplete PCR products are submitted to a modified procedure described in section b.

a) Nested PCR products containing complete ORFs

When the resulting nested PCR product contains the complete coding sequence, as predicted from the 5'EST sequence, it is cloned in an appropriate vector such as pED6dpc2, as described in section 3.

b) Nested PCR products containing incomplete ORFs

30

When the amplicon does not contain the complete coding sequence, intermediate steps are necessary to obtain both the complete coding sequence and a PCR product containing the full coding sequence. The complete coding sequence can be assembled from several partial sequences determined directly from different PCR products as described in the following section.

Once the full coding sequence has been completely determined, new primers compatible for PCR use are designed to obtain amplicons containing the whole coding region. However, in such cases, 3' primers compatible for PCR use are located inside the 3' UTR of the corresponding mRNA, thus yielding amplicons which lack part of this region, *i.e.* the polyA tract and sometimes the polyadenylation signal, as illustrated in figure 3. Such full length extended cDNAs are then cloned into an appropriate vector as described in section 3.

c) Sequencing extended cDNAs

Sequencing of extended cDNAs is performed using a Die Terminator approach with the AmpliTaq DNA polymerase FS kit available from Perkin Elmer.

In order to sequence PCR fragments, primer walking is performed using software such as OSP to choose primers and automated computer software such as ASMG (Sutton *et al.*, *Genome Science Technol.* 1: 9-19, 1995) to construct contigs of walking sequences including the initial 5' tag using minimum overlaps of 32 nucleotides. Preferably, primer walking is performed until the sequences of full length cDNAs are obtained.

Completion of the sequencing of a given extended cDNA fragment is assessed as follows. Since sequences located after a polyA tract are difficult to determine precisely in the case of uncloned products, sequencing and primer walking processes for PCR products are interrupted when a polyA tract is identified in extended cDNAs obtained as described in case b. The sequence length is compared to the size of the nested PCR product obtained as described above. Due to the limited accuracy of the determination of the PCR product size by gel electrophoresis, a sequence is considered complete if the size of the obtained sequence is at least 70 % the size of the first nested PCR product. If the length of the sequence determined from the computer analysis is not at least 70% of the length of the nested PCR product, these PCR products are cloned and the sequence of the insertion is determined. When Northern blot data are available, the size of the mRNA detected for a given PCR

product is used to finally assess that the sequence is complete. Sequences which do not fulfill the above criteria are discarded and will undergo a new isolation procedure.

Sequence data of all extended cDNAs are then transferred to a proprietary database, where quality controls and validation steps are carried out as described in
5 example 15.

3. Cloning of Full Length Extended cDNAs

The PCR product containing the full coding sequence is then cloned in an appropriate vector. For example, the extended cDNAs can be cloned into the expression vector
10 pED6dpc2 (DiscoverEase, Genetics Institute, Cambridge, MA) as follows. pED6dpc2 vector DNA is prepared with blunt ends by performing an EcoRI digestion followed by a fill in reaction. The blunt ended vector is dephosphorylated. After removal of PCR primers and ethanol precipitation, the PCR product containing the full coding sequence or the extended
15 cDNA obtained as described above is phosphorylated with a kinase subsequently removed by phenol-Sevag extraction and precipitation. The double stranded extended cDNA is then ligated to the vector and the resulting expression plasmid introduced into appropriate host cells.

Since the PCR products obtained as described above are blunt ended molecules that can be cloned in either direction, the orientation of several clones for each PCR product is
20 determined. Then, 4 to 10 clones are ordered in microtiter plates and subjected to a PCR reaction using a first primer located in the vector close to the cloning site and a second primer located in the portion of the extended cDNA corresponding to the 3' end of the mRNA. This second primer may be the antisense primer used in anchored PCR in the case of direct cloning (case a) or the antisense primer located inside the 3'UTR in the case of indirect cloning (case
25 b). Clones in which the start codon of the extended cDNA is operably linked to the promoter in the vector so as to permit expression of the protein encoded by the extended cDNA are conserved and sequenced. In addition to the ends of cDNA inserts, approximately 50 bp of vector DNA on each side of the cDNA insert are also sequenced.

The cloned PCR products are then entirely sequenced according to the
30 aforementioned procedure. In this case, contiguation of long fragments is then performed on walking sequences that have already contiguated for uncloned PCR products during

primer walking. Sequencing of cloned amplicons is complete when the resulting contigs include the whole coding region as well as overlapping sequences with vector DNA on both ends.

5 4. Computer analysis of Full Length Extended cDNA

Sequences of all full length extended cDNAs are then submitted to further analysis as described below. Before searching the extended full length cDNAs for sequences of interest, extended cDNAs which are not of interest (vector RNAs, transfer RNAs, ribosomal RNAs, mitochondrial RNAs, prokaryotic RNAs and fungal RNAs) are discarded using methods essentially similar to those described for 5'ESTs in Example 18.

10 *a) Identification of structural features*

Structural features, e.g. polyA tail and polyadenylation signal, of the sequences of full length extended cDNAs are subsequently determined as follows.

15 A polyA tail is defined as a homopolymeric stretch of at least 11 A with at most one alternative base within it. The polyA tail search is restricted to the last 100 nt of the sequence and limited to stretches of 11 consecutive A's because sequencing reactions are often not readable after such a polyA stretch. Stretches having more than 90% homology over 8 nucleotides are identified as polyA tails using BLAST2N.

20 To search for a polyadenylation signal, the polyA tail is clipped from the full-length sequence. The 50 bp preceding the polyA tail are first searched for the canonic polyadenylation AAUAAA signal and, if the canonic signal is not detected, for the alternative AUUAAA signal (Sheets *et al.*, *Nuc. Acids Res.* **18**: 5799-5805, 1990). If neither of these consensus polyadenylation signals is found, the canonic motif is searched again allowing one mismatch to account for possible sequencing errors. More than 85 %
25 of identified polyadenylation signals of either type actually ends 10 to 30 bp from the polyA tail. Alternative AUUAAA signals represents approximately 15 % of the total number of identified polyadenylation signals.

b) Identification of functional features

30 Functional features, e.g. ORFs and signal sequences, of the sequences of full length extended cDNAs were subsequently determined as follows.

The 3 upper strand frames of extended cDNAs are searched for ORFs defined as the maximum length fragments beginning with a translation initiation codon and ending with a stop codon. ORFs encoding at least 20 amino acids are preferred.

Each found ORF is then scanned for the presence of a signal peptide in the first 50 amino-acids or, where appropriate, within shorter regions down to 20 amino acids or less in the ORF, using the matrix method of von Heijne (*Nuc. Acids Res.* 14: 4683-4690, 1986), the disclosure of which is incorporated herein by reference as described in Example 22.

c) Homology to either nucleotidic or proteic sequences

Categorization of full-length sequences may be achieved using procedures essentially similar to those described for 5'ESTs in Example 24.

Extended cDNAs prepared as described above may be subsequently engineered to obtain nucleic acids which include desired portions of the extended cDNA using conventional techniques such as subcloning, PCR, or *in vitro* oligonucleotide synthesis. For example, nucleic acids which include only the full coding sequences (*i.e.* the sequences encoding the signal peptide and the mature protein remaining after the signal peptide is cleaved off) may be obtained using techniques known to those skilled in the art. Alternatively, conventional techniques may be applied to obtain nucleic acids which contain only the coding sequences for the mature protein remaining after the signal peptide is cleaved off or nucleic acids which contain only the coding sequences for the signal peptides.

Similarly, nucleic acids containing any other desired portion of the coding sequences for the secreted protein may be obtained. For example, the nucleic acid may contain at least 10 consecutive bases of an extended cDNA such as one of the extended cDNAs described below. In another embodiment, the nucleic acid may contain at least 15 consecutive bases of an extended cDNA such as one of the extended cDNAs described below. Alternatively, the nucleic acid may contain at least 20 consecutive bases of an extended cDNA such as one of the extended cDNAs described below. In another embodiment, the nucleic acid may contain at least 25 consecutive bases of an extended cDNA such as one of the extended cDNAs described below. In yet another embodiment, the nucleic acid may contain at least 40 consecutive bases of an extended cDNA such as one of the extended cDNAs described below.

Once an extended cDNA has been obtained, it can be sequenced to determine the amino acid sequence it encodes. Once the encoded amino acid sequence has been determined, one can create and identify any of the many conceivable cDNAs that will encode that protein by simply using the degeneracy of the genetic code. For example, allelic variants
5 or other homologous nucleic acids can be identified as described below. Alternatively, nucleic acids encoding the desired amino acid sequence can be synthesized *in vitro*.

In a preferred embodiment, the coding sequence may be selected using the known codon or codon pair preferences for the host organism in which the cDNA is to be expressed.

The extended cDNAs derived from the 5' ESTS of the present invention were
10 obtained as described in Example 28 below.

EXAMPLE 28

Characterization of cloned extended cDNAs obtained using 5' ESTs

The procedure described in Example 27 above was used to obtain the extended
15 cDNAs derived from the 5' ESTs of the present invention in a variety of tissues. The following list provides a few examples of thus obtained extended cDNAs.

Using this approach, the full length cDNA of SEQ ID NO:17 (internal identification number 48-19-3-G1-FL1) was obtained. This cDNA falls into the "EST-ext" category described above and encodes the signal peptide MKKVLLLLITAILAVAVG (SEQ ID NO:
20 18) having a von Heijne score of 8.2.

The full length cDNA of SEQ ID NO:19 (internal identification number 58-34-2-E7-FL2) was also obtained using this procedure. This cDNA falls into the "EST-ext" category described above and encodes the signal peptide MWWFQQGLSFLPSALVIWTS (SEQ ID NO:20) having a von Heijne score of 5.5.

25 Another full length cDNA obtained using the procedure described above has the sequence of SEQ ID NO:21 (internal identification number 51-27-1-E8-FL1). This cDNA, falls into the "EST-ext" category described above and encodes the signal peptide MVLTTLP SANSANSPVNMPPTTGPNLSYASSALSPCLT (SEQ ID NO:22) having a von Heijne score of 5.9.

30 The above procedure was also used to obtain a full length cDNA having the sequence of SEQ ID NO:23 (internal identification number 76-4-1-G5-FL1). This cDNA falls into the

"EST-ext" category described above and encodes the signal peptide ILSTVTALTFAXA (SEQ ID NO:24) having a von Heijne score of 5.5.

The full length cDNA of SEQ ID NO:25 (internal identification number 51-3-3-B10-FL3) was also obtained using this procedure. This cDNA falls into the "new" category described above and encodes a signal peptide LVLTLCTLPLAVA (SEQ ID NO:26) having a von Heijne score of 10.1.

The full length cDNA of SEQ ID NO:27 (internal identification number 58-35-2-F10-FL2) was also obtained using this procedure. This cDNA falls into the "new" category described above and encodes a signal peptide LWLLFFLVTAIHA (SEQ ID NO:28) having a von Heijne score of 10.7.

Bacterial clones containing plasmids containing the full length cDNAs described above are presently stored in the inventor's laboratories under the internal identification numbers provided above. The inserts may be recovered from the stored materials by growing an aliquot of the appropriate bacterial clone in the appropriate medium. The plasmid DNA can then be isolated using plasmid isolation procedures familiar to those skilled in the art such as alkaline lysis minipreps or large scale alkaline lysis plasmid isolation procedures. If desired the plasmid DNA may be further enriched by centrifugation on a cesium chloride gradient, size exclusion chromatography, or anion exchange chromatography. The plasmid DNA obtained using these procedures may then be manipulated using standard cloning techniques familiar to those skilled in the art. Alternatively, a PCR can be done with primers designed at both ends of the cDNA insertion. The PCR product which corresponds to the cDNA can then be manipulated using standard cloning techniques familiar to those skilled in the art.

The polypeptides encoded by the extended cDNAs may be screened for the presence of known structural or functional motifs or for the presence of signatures, small amino acid sequences which are well conserved amongst the members of a protein family. The conserved regions have been used to derive consensus patterns or matrices included in the PROSITE data bank, in particular in the file prosite.dat (Release 13.0 of November 1995, located at <http://expasy.hcuge.ch/sprot/prosite.html>). Prosit_convert and prosit_scan programs (http://ulrec3.unil.ch/ftpserveur/prosit_scan) may be used to find signatures on the extended cDNAs.

For each pattern obtained with the prosite_convert program from the prosite.dat file, the accuracy of the detection on a new protein sequence may be assessed by evaluating the frequency of irrelevant hits on the population of human secreted proteins included in the data bank SWISSPROT. The ratio between the number of hits on shuffled proteins (with a window size of 20 amino acids) and the number of hits on native (unshuffled) proteins may be used as an index. Every pattern for which the ratio is greater than 20% (one hit on shuffled proteins for 5 hits on native proteins) may be skipped during the search with prosite_scan. The program used to shuffle protein sequences (db_shuffled) and the program used to determine the statistics for each pattern in the protein data banks (prosite_statistics) are available on the ftp site http://ulrec3.unil.ch/ftpserveur/prosite_scan.

In addition to PCR based methods for obtaining extended cDNAs, traditional hybridization based methods may also be employed. These methods may also be used to obtain the genomic DNAs which encode the mRNAs from which the 5' ESTs were derived, mRNAs corresponding to the extended cDNAs, or nucleic acids which are homologous to extended cDNAs or 5' ESTs. Example 29 below provides examples of such methods.

EXAMPLE 29

Methods for Obtaining cDNAs which include the Entire Coding Region and the Authentic 5'End of the Corresponding mRNA

A full length cDNA library can be made using the strategies described in Examples 13, 14, 15, and 16 above by replacing the random nonamer used in Example 14 with an oligo-dT primer. For instance, the oligonucleotide of SEQ ID NO:14 may be used.

Alternatively, a cDNA library or genomic DNA library may be obtained from a commercial source or made using techniques familiar to those skilled in the art. Such cDNA or genomic DNA libraries may be used to isolate extended cDNAs obtained from 5' EST or nucleic acids homologous to extended cDNAs or 5' EST as follows. The cDNA library or genomic DNA library is hybridized to a detectable probe comprising at least 10 consecutive nucleotides from the 5' EST or extended cDNA using conventional techniques. Preferably, the probe comprises at least 12, 15, or 17 consecutive nucleotides from the 5' EST or extended cDNA. More preferably, the probe comprises at least 20 to 30 consecutive

nucleotides from the 5' EST or extended cDNA. In some embodiments, the probe comprises more than 30 nucleotides from the 5' EST or extended cDNA.

Techniques for identifying cDNA clones in a cDNA library which hybridize to a given probe sequence are disclosed in Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual* 2d Ed., Cold Spring Harbor Laboratory Press, 1989, the disclosure of which is incorporated herein by reference. The same techniques may be used to isolate genomic DNAs.

Briefly, cDNA or genomic DNA clones which hybridize to the detectable probe are identified and isolated for further manipulation as follows. A probe comprising at least 10 consecutive nucleotides from the 5' EST or extended cDNA is labeled with a detectable label such as a radioisotope or a fluorescent molecule. Preferably, the probe comprises at least 12, 15, or 17 consecutive nucleotides from the 5' EST or extended cDNA. More preferably, the probe comprises 20 to 30 consecutive nucleotides from the 5' EST or extended cDNA. In some embodiments, the probe comprises more than 30 nucleotides from the 5' EST or extended cDNA.

Techniques for labeling the probe are well known and include phosphorylation with polynucleotide kinase, nick translation, *in vitro* transcription, and non radioactive techniques. The cDNAs or genomic DNAs in the library are transferred to a nitrocellulose or nylon filter and denatured. After blocking of non specific sites, the filter is incubated with the labeled probe for an amount of time sufficient to allow binding of the probe to cDNAs or genomic DNAs containing a sequence capable of hybridizing thereto.

By varying the stringency of the hybridization conditions used to identify extended cDNAs or genomic DNAs which hybridize to the detectable probe, extended cDNAs having different levels of homology to the probe can be identified and isolated as described below.

1. Identification of Extended cDNA or Genomic cDNA Sequences Having a High Degree of Homology to the Labeled Probe

To identify extended cDNAs or genomic DNAs having a high degree of homology to the probe sequence, the melting temperature of the probe may be calculated using the following formulas:

For probes between 14 and 70 nucleotides in length the melting temperature (T_m) is calculated using the formula: $T_m = 81.5 + 16.6(\log [Na^+]) + 0.41(\text{fraction G+C}) - (600/N)$ where N is the length of the probe.

If the hybridization is carried out in a solution containing formamide, the melting temperature may be calculated using the equation $T_m = 81.5 + 16.6(\log [Na^+]) + 0.41(\text{fraction G+C}) - (0.63\% \text{ formamide}) - (600/N)$ where N is the length of the probe.

Prehybridization may be carried out in 6X SSC, 5X Denhardt's reagent, 0.5% SDS, 100 μ g denatured fragmented salmon sperm DNA or 6X SSC, 5X Denhardt's reagent, 0.5% SDS, 100 μ g denatured fragmented salmon sperm DNA, 50% formamide. The formulas for SSC and Denhardt's solutions are listed in Sambrook *et al.*, *supra*.

Hybridization is conducted by adding the detectable probe to the prehybridization solutions listed above. Where the probe comprises double stranded DNA, it is denatured before addition to the hybridization solution. The filter is contacted with the hybridization solution for a sufficient period of time to allow the probe to hybridize to extended cDNAs or genomic DNAs containing sequences complementary thereto or homologous thereto. For probes over 200 nucleotides in length, the hybridization may be carried out at 15-25°C below the T_m . For shorter probes, such as oligonucleotide probes, the hybridization may be conducted at 15-25°C below the T_m . Preferably, for hybridizations in 6X SSC, the hybridization is conducted at approximately 68°C. Preferably, for hybridizations in 50% formamide containing solutions, the hybridization is conducted at approximately 42°C.

All of the foregoing hybridizations would be considered to be under "stringent" conditions.

Following hybridization, the filter is washed in 2X SSC, 0.1% SDS at room temperature for 15 minutes. The filter is then washed with 0.1X SSC, 0.5% SDS at room temperature for 30 minutes to 1 hour. Thereafter, the solution is washed at the hybridization temperature in 0.1X SSC, 0.5% SDS. A final wash is conducted in 0.1X SSC at room temperature.

Extended cDNAs, nucleic acids homologous to extended cDNAs or 5' ESTs, or genomic DNAs which have hybridized to the probe are identified by autoradiography or other conventional techniques.

2. Obtention of Extended cDNA or Genomic cDNA Sequences Having Lower Degrees of Homology to the Labeled Probe

The above procedure may be modified to identify extended cDNAs, nucleic acids homologous to extended cDNAs, or genomic DNAs having decreasing levels of homology to the probe sequence. For example, to obtain extended cDNAs, nucleic acids homologous to extended cDNAs, or genomic DNAs of decreasing homology to the detectable probe, less stringent conditions may be used. For example, the hybridization temperature may be decreased in increments of 5°C from 68°C to 42°C in a hybridization buffer having a sodium concentration of approximately 1M. Following hybridization, the filter may be washed with 2X SSC, 0.5% SDS at the temperature of hybridization. These conditions are considered to be "moderate" conditions above 50°C and "low" conditions below 50°C.

Alternatively, the hybridization may be carried out in buffers, such as 6X SSC, containing formamide at a temperature of 42°C. In this case, the concentration of formamide in the hybridization buffer may be reduced in 5% increments from 50% to 0% to identify clones having decreasing levels of homology to the probe. Following hybridization, the filter may be washed with 6X SSC, 0.5% SDS at 50°C. These conditions are considered to be "moderate" conditions above 25% formamide and "low" conditions below 25% formamide.

Extended cDNAs, nucleic acids homologous to extended cDNAs, or genomic DNAs which have hybridized to the probe are identified by autoradiography.

3. Determination of the Degree of Homology Between the Obtained Extended cDNAs and the Labeled Probe

If it is desired to obtain nucleic acids homologous to extended cDNAs, such as allelic variants thereof or nucleic acids encoding proteins related to the proteins encoded by the extended cDNAs, the level of homology between the hybridized nucleic acid and the extended cDNA or 5' EST used as the probe may be further determined using BLAST2N; parameters may be adapted depending on the sequence length and degree of homology studied. To determine the level of homology between the hybridized nucleic acid and the extended cDNA or 5' EST from which the probe was derived, the nucleotide sequences of the hybridized nucleic acid and the extended cDNA or 5' EST from which the probe was derived are compared. For example, using the above methods, nucleic acids having at least 95%

nucleic acid homology to the extended cDNA or 5'EST from which the probe was derived may be obtained and identified. Similarly, by using progressively less stringent hybridization conditions one can obtain and identify nucleic acids having at least 90%, at least 85%, at least 80% or at least 75% homology to the extended cDNA or 5'EST from which the probe was
5 derived.

To determine whether a clone encodes a protein having a given amount of homology to the protein encoded by the extended cDNA or 5' EST, the amino acid sequence encoded by the extended cDNA or 5' EST is compared to the amino acid sequence encoded by the hybridizing nucleic acid. Homology is determined to exist when an amino acid sequence in
10 the extended cDNA or 5' EST is closely related to an amino acid sequence in the hybridizing nucleic acid. A sequence is closely related when it is identical to that of the extended cDNA or 5' EST or when it contains one or more amino acid substitutions therein in which amino acids having similar characteristics have been substituted for one another. Using the above methods and algorithms such as FASTA with parameters depending on the sequence length
15 and degree of homology studied, one can obtain nucleic acids encoding proteins having at least 95%, at least 90%, at least 85%, at least 80% or at least 75% homology to the proteins encoded by the extended cDNA or 5'EST from which the probe was derived.

In addition to the above described methods, other protocols are available to obtain
20 extended cDNAs using 5' ESTs as outlined in the following paragraphs.

Extended cDNAs may be prepared by obtaining mRNA from the tissue, cell, or organism of interest using mRNA preparation procedures utilizing polyA selection procedures or other techniques known to those skilled in the art. A first primer capable of hybridizing to the polyA tail of the mRNA is hybridized to the mRNA and a reverse
25 transcription reaction is performed to generate a first cDNA strand.

The first cDNA strand is hybridized to a second primer containing at least 10 consecutive nucleotides of the sequences of SEQ ID NOs 38-305. Preferably, the primer comprises at least 12, 15, or 17 consecutive nucleotides from the sequences of SEQ ID NOs 38-305. More preferably, the primer comprises 20 to 30 consecutive nucleotides from the
30 sequences of SEQ ID NOs 38-305. In some embodiments, the primer comprises more than 30 nucleotides from the sequences of SEQ ID NOs 38-305. If it is desired to obtain extended

cDNAs containing the full protein coding sequence, including the authentic translation initiation site, the second primer used contains sequences located upstream of the translation initiation site. The second primer is extended to generate a second cDNA strand complementary to the first cDNA strand. Alternatively, RT-PCR may be performed as described above using primers from both ends of the cDNA to be obtained.

Extended cDNAs containing 5' fragments of the mRNA may be prepared by hybridizing an mRNA comprising the sequence of the 5'EST for which an extended cDNA is desired with a primer comprising at least 10 consecutive nucleotides of the sequences complementary to the 5'EST and reverse transcribing the hybridized primer to make a first cDNA strand from the mRNAs. Preferably, the primer comprises at least 12, 15, or 17 consecutive nucleotides from the 5'EST. More preferably, the primer comprises 20 to 30 consecutive nucleotides from the 5'EST.

Thereafter, a second cDNA strand complementary to the first cDNA strand is synthesized. The second cDNA strand may be made by hybridizing a primer complementary to sequences in the first cDNA strand to the first cDNA strand and extending the primer to generate the second cDNA strand.

The double stranded extended cDNAs made using the methods described above are isolated and cloned. The extended cDNAs may be cloned into vectors such as plasmids or viral vectors capable of replicating in an appropriate host cell. For example, the host cell may be a bacterial, mammalian, avian, or insect cell.

Techniques for isolating mRNA, reverse transcribing a primer hybridized to mRNA to generate a first cDNA strand, extending a primer to make a second cDNA strand complementary to the first cDNA strand, isolating the double stranded cDNA and cloning the double stranded cDNA are well known to those skilled in the art and are described in *Current Protocols in Molecular Biology*, John Wiley and Sons, Inc. 1997 and Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, Second Edition, Cold Spring Harbor Laboratory Press, 1989, the entire disclosures of which are incorporated herein by reference.

Alternatively, procedures such as the one described in Example 29 may be used for obtaining full length cDNAs or extended cDNAs. In this approach, full length or extended cDNAs are prepared from mRNA and cloned into double stranded phagemids as follows. The cDNA library in the double stranded phagemids is then rendered single stranded by

treatment with an endonuclease, such as the Gene II product of the phage F1, and an exonuclease (Chang *et al.*, *Gene* 127:95-8, 1993). A biotinylated oligonucleotide comprising the sequence of a 5' EST, or a fragment containing at least 10 nucleotides thereof, is hybridized to the single stranded phagemids. Preferably, the fragment comprises at least 12, 15, or 17 consecutive nucleotides from the 5' EST. More preferably, the fragment comprises 20-30 consecutive nucleotides from the 5' EST. In some procedures, the fragment may comprise more than 30 consecutive nucleotides from the 5' EST.

Hybrids between the biotinylated oligonucleotide and phagemids having inserts containing the 5' EST sequence are isolated by incubating the hybrids with streptavidin coated paramagnetic beads and retrieving the beads with a magnet (Fry *et al.*, *Biotechniques*, 13: 124-131, 1992). Thereafter, the resulting phagemids containing the 5' EST sequence are released from the beads and converted into double stranded DNA using a primer specific for the 5' EST sequence. Alternatively, protocols such as the Gene Trapper kit (Gibco BRL) may be used. The resulting double stranded DNA is transformed into bacteria. Extended cDNAs containing the 5' EST sequence are identified by colony PCR or colony hybridization.

Using any of the above described methods in section III, a plurality of extended cDNAs containing full length protein coding sequences or sequences encoding only the mature protein remaining after the signal peptide is cleaved off may be provided as cDNA libraries for subsequent evaluation of the encoded proteins or use in diagnostic assays as described below.

IV. Expression of Proteins Encoded by Extended cDNAs Isolated Using 5' ESTs

Extended cDNAs containing the full protein coding sequences of their corresponding mRNAs or portions thereof, such as cDNAs encoding the mature protein, may be used to express the encoded secreted proteins or portions thereof as described in Example 30 below. If desired, the extended cDNAs may contain the sequences encoding the signal peptide to facilitate secretion of the expressed protein. It will be appreciated that a plurality of extended cDNAs containing the full protein coding sequences or portions thereof may be simultaneously cloned into expression vectors to create an expression library for analysis of the encoded proteins as described below.

EXAMPLE 30**Expression of the Proteins Encoded by the Genes Corresponding to 5'ESTS or Portions Thereof**

To express the proteins encoded by the genes corresponding to 5' ESTs (or portions thereof), full length cDNAs containing the entire protein coding region or extended cDNAs containing sequences adjacent to the 5' ESTs (or portions thereof) are obtained as described in Examples 27-29 and cloned into a suitable expression vector. If desired, the nucleic acids may contain the sequences encoding the signal peptide to facilitate secretion of the expressed protein. The nucleic acids inserted into the expression vectors may also contain sequences upstream of the sequences encoding the signal peptide, such as sequences which regulate expression levels or sequences which confer tissue specific expression.

The nucleic acid encoding the protein or polypeptide to be expressed is operably linked to a promoter in an expression vector using conventional cloning technology. The expression vector may be any of the mammalian, yeast, insect or bacterial expression systems known in the art. Commercially available vectors and expression systems are available from a variety of suppliers including Genetics Institute (Cambridge, MA), Stratagene (La Jolla, California), Promega (Madison, Wisconsin), and Invitrogen (San Diego, California). If desired, to enhance expression and facilitate proper protein folding, the codon context and codon pairing of the sequence may be optimized for the particular expression organism in which the expression vector is introduced, as explained by Hatfield, *et al.*, U.S. Patent No. 5,082,767, incorporated herein by this reference.

The cDNA cloned into the expression vector may encode the entire protein (*i.e.* the signal peptide and the mature protein), the mature protein (*i.e.* the protein created by cleaving the signal peptide off), only the signal peptide or any other portion thereof.

The following is provided as one exemplary method to express the proteins encoded by the extended cDNAs corresponding to the 5' ESTs or the nucleic acids described above. First, the methionine initiation codon for the gene and the polyA signal of the gene are identified. If the nucleic acid encoding the polypeptide to be expressed lacks a methionine to serve as the initiation site, an initiating methionine can be introduced next to the first codon of the nucleic acid using conventional techniques. Similarly, if the extended cDNA lacks a polyA signal, this sequence can be added to the construct by, for example, splicing out the

polyA signal from pSG5 (Stratagene) using BglII and SalI restriction endonuclease enzymes and incorporating it into the mammalian expression vector pXT1 (Stratagene). pXT1 contains the LTRs and a portion of the *gag* gene from Moloney Murine Leukemia Virus. The position of the LTRs in the construct allow efficient stable transfection. The vector
5 includes the Herpes Simplex thymidine kinase promoter and the selectable neomycin gene. The extended cDNA or portion thereof encoding the polypeptide to be expressed is obtained by PCR from the bacterial vector using oligonucleotide primers complementary to the extended cDNA or portion thereof and containing restriction endonuclease sequences for Pst I incorporated into the 5' primer and BglII at the 5' end of the corresponding cDNA 3' primer,
10 taking care to ensure that the extended cDNA is positioned with the poly A signal. The purified fragment obtained from the resulting PCR reaction is digested with PstI, blunt ended with an exonuclease, digested with Bgl II, purified and ligated to pXT1 containing a poly A signal and prepared for this ligation (blunt/BglII).

The ligated product is transfected into mouse NIH 3T3 cells using Lipofectin (Life
15 Technologies, Inc., Grand Island, New York) under conditions outlined in the product specification. Positive transfectants are selected after growing the transfected cells in 600 µg/ml G418 (Sigma, St. Louis, Missouri). Preferably the expressed protein is released into the culture medium, thereby facilitating purification.

Alternatively, the extended cDNAs may be cloned into pED6dpc2 as described
20 above. The resulting pED6dpc2 constructs may be transfected into a suitable host cell, such as COS 1 cells. Methotrexate resistant cells are selected and expanded. Preferably, the protein expressed from the extended cDNA is released into the culture medium thereby facilitating purification.

Proteins in the culture medium are separated by gel electrophoresis. If desired, the
25 proteins may be ammonium sulfate precipitated or separated based on size or charge prior to electrophoresis.

As a control, the expression vector lacking a cDNA insert is introduced into host cells or organisms and the proteins in the medium are harvested. The secreted proteins present in the medium are detected using techniques familiar to those skilled in the art such as
30 Coomassie blue or silver staining or using antibodies against the protein encoded by the extended cDNA.

Antibodies capable of specifically recognizing the protein of interest may be generated using synthetic 15-mer peptides having a sequence encoded by the appropriate 5' EST, extended cDNA, or portion thereof. The synthetic peptides are injected into mice to generate antibody to the polypeptide encoded by the 5' EST, extended cDNA, or portion thereof.

5 Secreted proteins from the host cells or organisms containing an expression vector which contains the extended cDNA derived from a 5' EST or a portion thereof are compared to those from the control cells or organism. The presence of a band in the medium from the cells containing the expression vector which is absent in the medium from the control cells indicates that the extended cDNA encodes a secreted protein. Generally, the band
10 corresponding to the protein encoded by the extended cDNA will have a mobility near that expected based on the number of amino acids in the open reading frame of the extended cDNA. However, the band may have a mobility different than that expected as a result of modifications such as glycosylation, ubiquitination, or enzymatic cleavage.

15 Alternatively, if the protein expressed from the above expression vectors does not contain sequences directing its secretion, the proteins expressed from host cells containing an expression vector with an insert encoding a secreted protein or portion thereof can be compared to the proteins expressed in control host cells containing the expression vector without an insert. The presence of a band in samples from cells containing the expression
20 vector with an insert which is absent in samples from cells containing the expression vector without an insert indicates that the desired protein or portion thereof is being expressed. Generally, the band will have the mobility expected for the secreted protein or portion thereof. However, the band may have a mobility different than that expected as a result of modifications such as glycosylation, ubiquitination, or enzymatic cleavage.

25 The protein encoded by the extended cDNA may be purified using standard immunochromatography techniques. In such procedures, a solution containing the secreted protein, such as the culture medium or a cell extract, is applied to a column having antibodies against the secreted protein attached to the chromatography matrix. The secreted protein is allowed to bind the immunochromatography column. Thereafter, the column is washed to remove non-specifically bound proteins. The specifically bound secreted protein is then
30 released from the column and recovered using standard techniques.

If antibody production is not possible, the extended cDNA sequence or portion thereof may be incorporated into expression vectors designed for use in purification schemes employing chimeric polypeptides. In such strategies, the coding sequence of the extended cDNA or portion thereof is inserted in frame with the gene encoding the other half of the chimera. The other half of the chimera may be β -globin or a nickel binding polypeptide. A chromatography matrix having antibody to β -globin or nickel attached thereto is then used to purify the chimeric protein. Protease cleavage sites may be engineered between the β -globin gene or the nickel binding polypeptide and the extended cDNA or portion thereof. Thus, the two polypeptides of the chimera may be separated from one another by protease digestion.

One useful expression vector for generating β -globin chimerics is pSG5 (Stratagene), which encodes rabbit β -globin. Intron II of the rabbit β -globin gene facilitates splicing of the expressed transcript, and the polyadenylation signal incorporated into the construct increases the level of expression. These techniques as described are well known to those skilled in the art of molecular biology. Standard methods are published in methods texts such as Davis *et al.*, (*Basic Methods in Molecular Biology*, Davis, Dibner, and Battey, ed., Elsevier Press, NY, 1986) and many of the methods are available from Stratagene, Life Technologies, Inc., or Promega. Polypeptide may additionally be produced from the construct using *in vitro* translation systems such as the *In vitro* ExpressTM Translation Kit (Stratagene).

Following expression and purification of the secreted proteins encoded by the 5' ESTs, extended cDNAs, or fragments thereof, the purified proteins may be tested for the ability to bind to the surface of various cell types as described in Example 31 below. It will be appreciated that a plurality of proteins expressed from these cDNAs may be included in a panel of proteins to be simultaneously evaluated for the activities specifically described below, as well as other biological roles for which assays for determining activity are available.

EXAMPLE 31

Analysis of Secreted Proteins to Determine Whether they Bind to the Cell Surface

The proteins encoded by the 5' ESTs, extended cDNAs, or fragments thereof are cloned into expression vectors such as those described in Example 30. The proteins are purified by size, charge, immunochromatography or other techniques familiar to those skilled

in the art. Following purification, the proteins are labeled using techniques known to those skilled in the art. The labeled proteins are incubated with cells or cell lines derived from a variety of organs or tissues to allow the proteins to bind to any receptor present on the cell surface. Following the incubation, the cells are washed to remove non-specifically bound protein. The labeled proteins are detected by autoradiography. Alternatively, unlabeled proteins may be incubated with the cells and detected with antibodies having a detectable label, such as a fluorescent molecule, attached thereto.

Specificity of cell surface binding may be analyzed by conducting a competition analysis in which various amounts of unlabeled protein are incubated along with the labeled protein. The amount of labeled protein bound to the cell surface decreases as the amount of competitive unlabeled protein increases. As a control, various amounts of an unlabeled protein unrelated to the labeled protein is included in some binding reactions. The amount of labeled protein bound to the cell surface does not decrease in binding reactions containing increasing amounts of unrelated unlabeled protein, indicating that the protein encoded by the cDNA binds specifically to the cell surface.

As discussed above, secreted proteins have been shown to have a number of important physiological effects and, consequently, represent a valuable therapeutic resource. The secreted proteins encoded by the extended cDNAs or portions thereof made according to Examples 27-29 may be evaluated to determine their physiological activities as described below.

EXAMPLE 32

Assaying the Proteins Expressed from Extended cDNAs or Portions Thereof for Cytokine, Cell Proliferation or Cell Differentiation Activity

As discussed above, secreted proteins may act as cytokines or may affect cellular proliferation or differentiation. Many protein factors discovered to date, including all known cytokines, have exhibited activity in one or more factor dependent cell proliferation assays, and hence the assays serve as a convenient confirmation of cytokine activity. The activity of a protein encoded by the extended cDNAs is evidenced by any one of a number of routine factor dependent cell proliferation assays for cell lines including, without limitation, 32D,

DA2, DA1G, T10, B9, B9/11, BaF3, MC9/G, M⁺ (preB M⁺), 2E8, RB5, DA1, 123, T1165, HT2, CTLL2, TF-1, Mo7c and CMK. The proteins encoded by the above extended cDNAs or portions thereof may be evaluated for their ability to regulate T cell or thymocyte proliferation in assays such as those described above or in the following references, which are
5 incorporated herein by reference: *Current Protocols in Immunology*, Ed. by Coligan *et al.*, Greene Publishing Associates and Wiley-Interscience; Takai *et al. J. Immunol.* 137:3494-3500, 1986., Bertagnolli *et al.*, *J. Immunol.* 145:1706-1712, 1990., Bertagnolli *et al.*, *Cell. Immunol.* 133:327-341, 1991; Bertagnolli, *et al.*, *J. Immunol.* 149:3778-3783, 1992; Bowman *et al.*, *J. Immunol.* 152:1756-1761, 1994.

10 In addition, numerous assays for cytokine production and/or the proliferation of spleen cells, lymph node cells and thymocytes are known. These include the techniques disclosed in *Current Protocols in Immunology*, *supra* 1:3.12.1-3.12.14; and Schreiber In *Current Protocols in Immunology*, *supra* 1 : 6.8.1-6.8.8.

The proteins encoded by the cDNAs may also be assayed for the ability to regulate
15 the proliferation and differentiation of hematopoietic or lymphopoietic cells. Many assays for such activity are familiar to those skilled in the art, including the assays in the following references, which are incorporated herein by reference: Bottomly *et al.*, In *Current Protocols in Immunology*, *supra* 1 : 6.3.1-6.3.12.; deVries *et al.*, *J. Exp. Med.* 173:1205-1211, 1991; Moreau *et al.*, *Nature* 36:690-692, 1988; Greenberger *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*
20 80:2931-2938, 1983; Nordan, R., In *Current Protocols in Immunology*, *supra* 1 : 6.6.1-6.6.5; Smith *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 83:1857-1861, 1986; Bennett *et al.*, in *Current Protocols in Immunology supra* 1 : 6.15.1; Ciarletta *et al.*, In *Current Protocols in Immunology. supra* 1 : 6.13.1.

The proteins encoded by the cDNAs may also be assayed for their ability to regulate
25 T-cell responses to antigens. Many assays for such activity are familiar to those skilled in the art, including the assays described in the following references, which are incorporated herein by reference: Chapter 3 (*In Vitro* Assays for Mouse Lymphocyte Function), Chapter 6 (Cytokines and Their Cellular Receptors) and Chapter 7, (Immunologic Studies in Humans) in *Current Protocols in Immunology supra*; Weinberger *et al.*, *Proc. Natl. Acad. Sci. USA*
30 77:6091-6095, 1980; Weinberger *et al.*, *Eur. J. Immun.* 11:405-411, 1981; Takai *et al.*, *J. Immunol.* 137:3494-3500, 1986; Takai *et al.*, *J. Immunol.* 140:508-512, 1988.

Those proteins which exhibit cytokine, cell proliferation, or cell differentiation activity may then be formulated as pharmaceuticals and used to treat clinical conditions in which induction of cell proliferation or differentiation is beneficial. Alternatively, as described in more detail below, genes encoding these proteins or nucleic acids regulating the expression of these proteins may be introduced into appropriate host cells to increase or decrease the expression of the proteins as desired.

EXAMPLE 33

Assaying the Proteins Expressed from Extended cDNAs or Portions

Thereof for Activity as Immune System Regulators

The proteins encoded by the cDNAs may also be evaluated for their effects as immune regulators. For example, the proteins may be evaluated for their activity to influence thymocyte or splenocyte cytotoxicity. Numerous assays for such activity are familiar to those skilled in the art including the assays described in the following references, which are incorporated herein by reference: Chapter 3 (*In Vitro* Assays for Mouse Lymphocyte Function 3.1-3.19) and Chapter 7 (Immunologic studies in Humans) in *Current Protocols in Immunology*, Coligan *et al.*, Eds, Greene Publishing Associates and Wiley-Interscience; Herrmann *et al.*, *Proc. Natl. Acad. Sci. USA* 78:2488-2492, 1981; Herrmann *et al.*, *J. Immunol.* 128:1968-1974, 1982; Handa *et al.*, *J. Immunol.* 135:1564-1572, 1985; Takai *et al.*, *J. Immunol.* 137:3494-3500, 1986; Takai *et al.*, *J. Immunol.* 140:508-512, 1988; Bowman *et al.*, *J. Virology* 61:1992-1998; Bertagnolli *et al.*, *Cell. Immunol.* 133:327-341, 1991; Brown *et al.*, *J. Immunol.* 153:3079-3092, 1994.

The proteins encoded by the cDNAs may also be evaluated for their effects on T-cell dependent immunoglobulin responses and isotype switching. Numerous assays for such activity are familiar to those skilled in the art, including the assays disclosed in the following references, which are incorporated herein by reference: Maliszewski, *J. Immunol.* 144:3028-3033, 1990; Mond *et al.* in *Current Protocols in Immunology*, 1 : 3.8.1-3.8.16, *supra*.

The proteins encoded by the cDNAs may also be evaluated for their effect on immune effector cells, including their effect on Th1 cells and cytotoxic lymphocytes. Numerous assays for such activity are familiar to those skilled in the art, including the assays disclosed in the following references, which are incorporated herein by reference: Chapter 3 (*In Vitro* Assays

for Mouse Lymphocyte Function 3.1-3.19) and Chapter 7 (Immunologic Studies in Humans) in *Current Protocols in Immunology*, *supra*; Takai *et al.*, *J. Immunol.* **137**:3494-3500, 1986; Takai *et al.*, *J. Immunol.* **140**:508-512, 1988; Bertagnolli *et al.*, *J. Immunol.* **149**:3778-3783, 1992.

- 5 The proteins encoded by the cDNAs may also be evaluated for their effect on dendritic cell mediated activation of naive T-cells. Numerous assays for such activity are familiar to those skilled in the art, including the assays disclosed in the following references, which are incorporated herein by reference: Guery *et al.*, *J. Immunol.* **134**:536-544, 1995; Inaba *et al.*, *J. Exp. Med.* **173**:549-559, 1991; Macatonia *et al.*, *J. Immunol.* **154**:5071-5079, 1995; Porgador *et al.*, *J. Exp. Med.* **182**:255-260, 1995; Nair *et al.*, *J. Virol.* **67**:4062-4069, 1993; Huang *et al.*, *Science* **264**:961-965, 1994; Macatonia *et al.*, *J. Exp. Med.* **169**:1255-1264, 1989; Bhardwaj *et al.*, *Journal of Clinical Investigation* **94**:797-807, 1994; and Inaba *et al.*, *J. Exp. Med.* **172**:631-640, 1990.

- 15 The proteins encoded by the cDNAs may also be evaluated for their influence on the lifetime of lymphocytes. Numerous assays for such activity are familiar to those skilled in the art, including the assays disclosed in the following references, which are incorporated herein by reference: Darzynkiewicz *et al.*, *Cytometry* **13**:795-808, 1992; Gorczyca *et al.*, *Leukemia* **7**:659-670, 1993; Gorczyca *et al.*, *Cancer Res.* **53**:1945-1951, 1993; Itoh *et al.*, *Cell* **66**:233-243, 1991; Zacharchuk, *J. Immunol.* **145**:4037-4045, 1990; Zarnai *et al.*, *Cytometry* **14**:891-897, 1993; Gorczyca *et al.*, *Int. J. Oncol.* **1**:639-648, 1992.

- 25 The proteins encoded by the cDNAs may also be evaluated for their influence on early steps of T-cell commitment and development. Numerous assays for such activity are familiar to those skilled in the art, including without limitation the assays disclosed in the following references, which are incorporated herein by references: Antica *et al.*, *Blood* **84**:111-117, 1994; Fine *et al.*, *Cell. Immunol.* **155**:111-122, 1994; Galy *et al.*, *Blood* **85**:2770-2778, 1995; Toki *et al.*, *Proc. Nat. Acad. Sci. USA* **88**:7548-7551, 1991.

- 30 Those proteins which exhibit activity as immune system regulators activity may then be formulated as pharmaceuticals and used to treat clinical conditions in which regulation of immune activity is beneficial. For example, the protein may be useful in the treatment of various immune deficiencies and disorders (including severe combined immunodeficiency), e.g., in regulating (up or down) growth and proliferation of T and/or B lymphocytes, as well

as effecting the cytolytic activity of NK cells and other cell populations. These immune deficiencies may be genetic or be caused by viral (e.g., HIV) as well as bacterial or fungal infections, or may result from autoimmune disorders. More specifically, infectious diseases caused by viral, bacterial, fungal or other infection may be treatable using a protein encoded by extended cDNAs derived from the 5' ESTs of the present invention, including infections by HIV, hepatitis viruses, herpesviruses, mycobacteria, Leishmania spp., plamodium and various fungal infections such as candidiasis. Of course, in this regard, a protein encoded by extended cDNAs derived from the 5' ESTs of the present invention may also be useful where a boost to the immune system generally may be desirable, *i.e.*, in the treatment of cancer.

Alternatively, proteins encoded by extended cDNAs derived from the 5' ESTs of the present invention may be used in treatment of autoimmune disorders including, for example, connective tissue disease, multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, autoimmune pulmonary inflammation, Guillain-Barre syndrome, autoimmune thyroiditis, insulin dependent diabetes mellitis, myasthenia gravis, graft-versus-host disease and autoimmune inflammatory eye disease. Such a protein encoded by extended cDNAs derived from the 5' ESTs of the present invention may also to be useful in the treatment of allergic reactions and conditions, such as asthma (particularly allergic asthma) or other respiratory problems. Other conditions, in which immune suppression is desired (including, for example, organ transplantation), may also be treatable using a protein encoded by extended cDNAs derived from the 5' ESTs of the present invention.

Using the proteins of the invention it may also be possible to regulate immune responses either up or down.

Down regulation may involve inhibiting or blocking an immune response already in progress or may involve preventing the induction of an immune response. The functions of activated T-cells may be inhibited by suppressing T cell responses or by inducing specific tolerance in T cells, or both. Immunosuppression of T cell responses is generally an active non-antigen-specific process which requires continuous exposure of the T cells to the suppressive agent. Tolerance, which involves inducing non-responsiveness or anergy in T cells, is distinguishable from immunosuppression in that it is generally antigen-specific and persists after the end of exposure to the tolerizing agent. Operationally, tolerance can be

demonstrated by the lack of a T cell response upon reexposure to specific antigen in the absence of the tolerizing agent.

Down regulating or preventing one or more antigen functions (including without limitation B lymphocyte antigen functions, such as, for example, B7 costimulation), e.g., preventing high level lymphokine synthesis by activated T cells, will be useful in situations of tissue, skin and organ transplantation and in graft-versus-host disease (GVHD). For example, blockage of T cell function should result in reduced tissue destruction in tissue transplantation. Typically, in tissue transplants, rejection of the transplant is initiated through its recognition as foreign by T cells, followed by an immune reaction that destroys the transplant. The administration of a molecule which inhibits or blocks interaction of a B7 lymphocyte antigen with its natural ligand(s) on immune cells (such as a soluble, monomeric form of a peptide having B7-2 activity alone or in conjunction with a monomeric form of a peptide having an activity of another B lymphocyte antigen (e.g., B7-1, B7-3) or blocking antibody), prior to transplantation, can lead to the binding of the molecule to the natural ligand(s) on the immune cells without transmitting the corresponding costimulatory signal. Blocking B lymphocyte antigen function in this manner prevents cytokine synthesis by immune cells, such as T cells, and thus acts as an immunosuppressant. Moreover, the lack of costimulation may also be sufficient to anergize the T cells, thereby inducing tolerance in a subject. Induction of long-term tolerance by B lymphocyte antigen-blocking reagents may avoid the necessity of repeated administration of these blocking reagents. To achieve sufficient immunosuppression or tolerance in a subject, it may also be necessary to block the function of a combination of B lymphocyte antigens.

The efficacy of particular blocking reagents in preventing organ transplant rejection or GVHD can be assessed using animal models that are predictive of efficacy in humans. Examples of appropriate systems which can be used include allogeneic cardiac grafts in rats and xenogeneic pancreatic islet cell grafts in mice, both of which have been used to examine the immunosuppressive effects of CTLA4Ig fusion proteins *in vivo* as described in Lenschow *et al.*, *Science* 257:789-792, 1992 and Turka *et al.*, *Proc. Natl. Acad. Sci USA*, 89:11102-11105, 1992. In addition, murine models of GVHD (see Paul ed., *Fundamental Immunology*, Raven Press, New York, 1989, pp. 846-847) can be used to determine the effect of blocking B lymphocyte antigen function *in vivo* on the development of that disease.

Blocking antigen function may also be therapeutically useful for treating autoimmune diseases. Many autoimmune disorders are the result of inappropriate activation of T cells that are reactive against self tissue and which promote the production of cytokines and autoantibodies involved in the pathology of the diseases. Preventing the activation of autoreactive T cells may reduce or eliminate disease symptoms. Administration of reagents which block costimulation of T cells by disrupting receptor/ligand interactions of B lymphocyte antigens can be used to inhibit T cell activation and prevent production of autoantibodies or T cell-derived cytokines which potentially involved in the disease process. Additionally, blocking reagents may induce antigen-specific tolerance of autoreactive T cells which could lead to long-term relief from the disease. The efficacy of blocking reagents in preventing or alleviating autoimmune disorders can be determined using a number of well-characterized animal models of human autoimmune diseases. Examples include murine experimental autoimmune encephalitis, systemic lupus erythmatosis in MRL/pr/pr mice or NZB hybrid mice, murine autoimmuno collagen arthritis, diabetes mellitus in OD mice and BB rats, and murine experimental myasthenia gravis (see Paul ed., *supra*, pp. 840-856).

Upregulation of an antigen function (preferably a B lymphocyte antigen function), as a means of up regulating immune responses, may also be useful in therapy. Upregulation of immune responses may involve either enhancing an existing immune response or eliciting an initial immune response as shown by the following examples. For instance, enhancing an immune response through stimulating B lymphocyte antigen function may be useful in cases of viral infection. In addition, systemic viral diseases such as influenza, the common cold, and encephalitis might be alleviated by the administration of stimulatory form of B lymphocyte antigens systemically.

Alternatively, antiviral immune responses may be enhanced in an infected patient by removing T cells from the patient, costimulating the T cells *in vitro* with viral antigen-pulsed APCs either expressing a peptide encoded by extended cDNAs derived from the 5' ESTs of the present invention or together with a stimulatory form of a soluble peptide encoded by extended cDNAs derived from the 5' ESTs of the present invention and reintroducing the *in vitro* primed T cells into the patient. The infected cells would now be capable of delivering a costimulatory signal to T cells *in vivo*, thereby activating the T cells.

In another application, upregulation or enhancement of antigen function (preferably B lymphocyte antigen function) may be useful in the induction of tumor immunity. Tumor cells (e.g., sarcoma, melanoma, lymphoma, leukemia, neuroblastoma, carcinoma) transfected with a nucleic acid encoding at least one peptide encoded by extended cDNAs derived from the 5' ESTs of the present invention can be administered to a subject to overcome tumor-specific tolerance in the subject. If desired, the tumor cell can be transfected to express a combination of peptides. For example, tumor cells obtained from a patient can be transfected *ex vivo* with an expression vector directing the expression of a peptide having B7-2-like activity alone, or in conjunction with a peptide having B7-1-like activity and/or B7-3-like activity. The transfected tumor cells are returned to the patient to result in expression of the peptides on the surface of the transfected cell. Alternatively, gene therapy techniques can be used to target a tumor cell for transfection *in vivo*.

The presence of the peptide encoded by extended cDNAs derived from the 5' ESTs of the present invention having the activity of a B lymphocyte antigen(s) on the surface of the tumor cell provides the necessary costimulation signal to T cells to induce a T cell mediated immune response against the transfected tumor cells. In addition, tumor cells which lack or which fail to reexpress sufficient amounts of MHC class I or MHC class II molecules can be transfected with nucleic acids encoding all or a portion of (e.g., a cytoplasmic-domain truncated portion) of an MHC class I α chain and β_2 microglobulin or an MHC class II α chain and an MHC class II β chain to thereby express MHC class I or MHC class II proteins on the cell surface, respectively. Expression of the appropriate MHC class I or class II molecules in conjunction with a peptide having the activity of a B lymphocyte antigen (e.g., B7-1, B7-2, B7-3) induces a T cell mediated immune response against the transfected tumor cell. Optionally, a gene encoding an antisense construct which blocks expression of an MHC class II associated protein, such as the invariant chain, can also be cotransfected with a DNA encoding a peptide having the activity of a B lymphocyte antigen to promote presentation of tumor associated antigens and induce tumor specific immunity. Thus, the induction of a T cell mediated immune response in a human subject may be sufficient to overcome tumor-specific tolerance in the subject. Alternatively, as described in more detail below, genes encoding these immune system regulator proteins or nucleic acids regulating the expression of

such proteins may be introduced into appropriate host cells to increase or decrease the expression of the proteins as desired.

EXAMPLE 34

5

Assaying the Proteins Expressed from Extended cDNAs or Portions Thereof for Hematopoiesis Regulating Activity

The proteins encoded by the extended cDNAs or portions thereof may also be evaluated for their hematopoiesis regulating activity. For example, the effect of the proteins on embryonic stem cell differentiation may be evaluated. Numerous assays for such activity are familiar to those skilled in the art, including the assays disclosed in the following references, which are incorporated herein by reference: Johansson *et al.* *Cell. Biol.* 15:141-151, 1995; Keller *et al.*, *Mol. Cell. Biol.* 13:473-486, 1993; McClanahan *et al.*, *Blood* 81:2903-2915, 1993.

The proteins encoded by the extended cDNAs or portions thereof may also be evaluated for their influence on the lifetime of stem cells and stem cell differentiation. Numerous assays for such activity are familiar to those skilled in the art, including the assays disclosed in the following references, which are incorporated herein by reference: Freshney, Methylcellulose Colony Forming Assays, in *Culture of Hematopoietic Cells.*, Freshney, *et al.* Eds. pp. 265-268, Wiley-Liss, Inc., New York, NY. 1994; Hirayama *et al.*, *Proc. Natl. Acad. Sci. USA* 89:5907-5911, 1992; McNiece and Briddell, in *Culture of Hematopoietic Cells, supra*; Neben *et al.*, *Exp. Hematol.* 22:353-359, 1994; Ploemacher and Cobblestone In *Culture of Hematopoietic Cells, supra* 1-21, Spooncer *et al.*, in *Culture of Hematopoietic Cells, supra* 163-179 and Sutherland in *Culture of Hematopoietic Cells, supra*. 139-162.

Those proteins which exhibit hematopoiesis regulatory activity may then be formulated as pharmaceuticals and used to treat clinical conditions in which regulation of hematopoiesis is beneficial, such as in the treatment of myeloid or lymphoid cell deficiencies. Involvement in regulating hematopoiesis is indicated even by marginal biological activity in support of colony forming cells or of factor-dependent cell lines. For example, proteins supporting the growth and proliferation of erythroid progenitor cells alone or in combination with other cytokines, indicates utility, for example, in treating various anemias or for use in conjunction with irradiation/chemotherapy to stimulate the production of erythroid precursors

and/or erythroid cells. Proteins supporting the growth and proliferation of myeloid cells such as granulocytes and monocytes/macrophages (*i.e.*, traditional CSF activity) may be useful, for example, in conjunction with chemotherapy to prevent or treat consequent myelo-suppression. Proteins supporting the growth and proliferation of megakaryocytes and consequently of platelets allows prevention or treatment of various platelet disorders such as thrombocytopenia, and generally may be used in place of or complementary to platelet transfusions. Proteins supporting the growth and proliferation of hematopoietic stem cells which are capable of maturing to any and all of the above-mentioned hematopoietic cells may therefore find therapeutic utility in various stem cell disorders (such as those usually treated with transplantation, including, without limitation, aplastic anemia and paroxysmal nocturnal hemoglobinuria), as well as in repopulating the stem cell compartment post irradiation/chemotherapy, either *in vivo* or *ex vivo* (*i.e.*, in conjunction with bone marrow transplantation or with peripheral progenitor cell transplantation (homologous or heterologous)) as normal cells or genetically manipulated for gene therapy. Alternatively, as described in more detail below, genes encoding hematopoiesis regulating activity proteins or nucleic acids regulating the expression of such proteins may be introduced into appropriate host cells to increase or decrease the expression of the proteins as desired.

EXAMPLE 35

Assaying the Proteins Expressed from Extended cDNAs or Portions Thereof for Regulation of Tissue Growth

The proteins encoded by the extended cDNAs or portions thereof may also be evaluated for their effect on tissue growth. Numerous assays for such activity are familiar to those skilled in the art, including the assays disclosed in International Patent Publication No. WO95/16035, International Patent Publication No. WO95/05846 and International Patent Publication No. WO91/07491, which are incorporated herein by reference.

Assays for wound healing activity include, without limitation, those described in: Winter, *Epidermal Wound Healing*, pps. 71-112, Maibach and Rovee, eds., Year Book Medical Publishers, Inc., Chicago, as modified by Eaglstein and Mertz, *J. Invest. Dermatol.* 71:382-84, 1978, which are incorporated herein by reference.

Those proteins which are involved in the regulation of tissue growth may then be formulated as pharmaceuticals and used to treat clinical conditions in which regulation of tissue growth is beneficial. For example, a protein encoded by extended cDNAs derived from the 5' ESTs of the present invention also may have utility in compositions used for bone, cartilage, tendon, ligament and/or nerve tissue growth or regeneration, as well as for wound healing and tissue repair and replacement, and in the treatment of burns, incisions and ulcers.

A protein encoded by extended cDNAs derived from the 5' ESTs of the present invention, which induces cartilage and/or bone growth in circumstances where bone is not normally formed, has application in the healing of bone fractures and cartilage damage or defects in humans and other animals. Such a preparation employing a protein of the invention may have prophylactic use in closed as well as open fracture reduction and also in the improved fixation of artificial joints. *De novo* bone synthesis induced by an osteogenic agent contributes to the repair of congenital, trauma induced, or oncologic resection induced craniofacial defects, and also is useful in cosmetic plastic surgery.

A protein of this invention may also be used in the treatment of periodontal disease, and in other tooth repair processes. Such agents may provide an environment to attract bone-forming cells, stimulate growth of bone-forming cells or induce differentiation of bone-forming cell progenitors. A protein of the invention may also be useful in the treatment of osteoporosis or osteoarthritis, such as through stimulation of bone and/or cartilage repair or by blocking inflammation or processes of tissue destruction (collagenase activity, osteoclast activity, etc.) mediated by inflammatory processes.

Another category of tissue regeneration activity that may be attributable to the protein encoded by extended cDNAs derived from the 5' ESTs of the present invention is tendon/ligament formation. A protein encoded by extended cDNAs derived from the 5' ESTs of the present invention, which induces tendon/ligament-like tissue or other tissue formation in circumstances where such tissue is not normally formed, has application in the healing of tendon or ligament tears, deformities and other tendon or ligament defects in humans and other animals. Such a preparation employing a tendon/ligament-like tissue inducing protein may have prophylactic use in preventing damage to tendon or ligament tissue, as well as use in the improved fixation of tendon or ligament to bone or other tissues, and in repairing defects to tendon or ligament tissue. *De novo* tendon/ligament-like tissue

formation induced by a composition encoded by extended cDNAs derived from the 5' ESTs of the present invention contributes to the repair of tendon or ligaments defects of congenital, traumatic or other origin and is also useful in cosmetic plastic surgery for attachment or repair of tendons or ligaments. The compositions encoded by extended cDNAs derived from the 5' ESTs of the present invention may provide an environment to attract tendon- or ligament-forming cells, stimulate growth of tendon- or ligament-forming cells, induce differentiation of progenitors of tendon- or ligament-forming cells, or induce growth of tendon/ligament cells or progenitors *ex vivo* for return *in vivo* to effect tissue repair. The compositions of the invention may also be useful in the treatment of tendinitis, carpal tunnel syndrome and other tendon or ligament defects. The compositions may also include an appropriate matrix and/or sequestering agent as a carrier as is well known in the art.

The protein encoded by extended cDNAs derived from the 5' ESTs of the present invention may also be useful for proliferation of neural cells and for regeneration of nerve and brain tissue, *i.e.*, for the treatment of central and peripheral nervous system diseases and neuropathies, as well as mechanical and traumatic disorders, which involve degeneration, death or trauma to neural cells or nerve tissue. More specifically, a protein may be used in the treatment of diseases of the peripheral nervous system, such as peripheral nerve injuries, peripheral neuropathy and localized neuropathies, and central nervous system diseases, such as Alzheimer's, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and Shy-Drager syndrome. Further conditions which may be treated in accordance with the present invention include mechanical and traumatic disorders, such as spinal cord disorders, head trauma and cerebrovascular diseases such as stroke. Peripheral neuropathies resulting from chemotherapy or other medical therapies may also be treatable using a protein of the invention.

Proteins of the invention may also be useful to promote better or faster closure of non-healing wounds, including without limitation pressure ulcers, ulcers associated with vascular insufficiency, surgical and traumatic wounds, and the like.

It is expected that a protein encoded by extended cDNAs derived from the 5' ESTs of the present invention may also exhibit activity for generation or regeneration of other tissues, such as organs (including, for example, pancreas, liver, intestine, kidney, skin, endothelium) muscle (smooth, skeletal or cardiac) and vascular (including vascular

endothelium) tissue, or for promoting the growth of cells comprising such tissues. Part of the desired effects may be by inhibition or modulation of fibrotic scarring to allow normal tissue to generate. A protein of the invention may also exhibit angiogenic activity.

5 A protein encoded by extended cDNAs derived from the 5' ESTs of the present invention may also be useful for gut protection or regeneration and treatment of lung or liver fibrosis, reperfusion injury in various tissues, and conditions resulting from systemic cytokine damage.

10 A protein encoded by extended cDNAs derived from the 5' ESTs of the present invention may also be useful for promoting or inhibiting differentiation of tissues described above from precursor tissues or cells; or for inhibiting the growth of tissues described above.

Alternatively, as described in more detail below, genes encoding tissue growth regulating activity proteins or nucleic acids regulating the expression of such proteins may be introduced into appropriate host cells to increase or decrease the expression of the proteins as desired.

15

EXAMPLE 36

Assaying the Proteins Expressed from Extended cDNAs or Portions

Thereof for Regulation of Reproductive Hormones

20 The proteins encoded by the extended cDNAs or portions thereof may also be evaluated for their ability to regulate reproductive hormones, such as follicle stimulating hormone. Numerous assays for such activity are familiar to those skilled in the art, including the assays disclosed in the following references, which are incorporated herein by reference: Vale *et al.*, *Endocrinol.* **91**:562-572, 1972; Ling *et al.*, *Nature* **321**:779-782, 1986; Vale *et al.*, *Nature* **321**:776-779, 1986; Mason *et al.*, *Nature* **318**:659-663, 1985; Forage *et al.*,
25 *Proc. Natl. Acad. Sci. USA* **83**:3091-3095, 1986, Chapter 6.12 in *Current Protocols in Immunology*, Coligan *et al.* Eds. Greene Publishing Associates and Wiley-Interscience; Taub *et al.*, *J. Clin. Invest.* **95**:1370-1376, 1995; Lind *et al.*, *APMIS* **103**:140-146, 1995; Muller *et al.*, *Eur. J. Immunol.* **25**:1744-1748; Gruber *et al.*, *J. Immunol.* **152**:5860-5867, 1994; Johnston *et al.*, *J Immunol.* **153**:1762-1768, 1994.

30

Those proteins which exhibit activity as reproductive hormones or regulators of cell movement may then be formulated as pharmaceuticals and used to treat clinical conditions in

which regulation of reproductive hormones are beneficial. For example, a protein encoded by extended cDNAs derived from the 5' ESTs of the present invention may also exhibit activin- or inhibin-related activities. Inhibins are characterized by their ability to inhibit the release of follicle stimulating hormone (FSH), while activins are characterized by their ability to stimulate the release of FSH. Thus, a protein encoded by extended cDNAs derived from the 5' ESTs of the present invention, alone or in heterodimers with a member of the inhibin α family, may be useful as a contraceptive based on the ability of inhibins to decrease fertility in female mammals and decrease spermatogenesis in male mammals. Administration of sufficient amounts of other inhibins can induce infertility in these mammals. Alternatively, the protein of the invention, as a homodimer or as a heterodimer with other protein subunits of the inhibin-B group, may be useful as a fertility inducing therapeutic, based upon the ability of activin molecules in stimulating FSH release from cells of the anterior pituitary. See, for example, United States Patent 4,798,885, the disclosure of which is incorporated herein by reference. A protein of the invention may also be useful for advancement of the onset of fertility in sexually immature mammals, so as to increase the lifetime reproductive performance of domestic animals such as cows, sheep and pigs.

Alternatively, as described in more detail below, genes encoding reproductive hormone regulating activity proteins or nucleic acids regulating the expression of such proteins may be introduced into appropriate host cells to increase or decrease the expression of the proteins as desired.

EXAMPLE 37

Assaying the Proteins Expressed from Extended cDNAs or Portions Thereof for Chemotactic/Chemokinetic Activity

The proteins encoded by the extended cDNAs or portions thereof may also be evaluated for chemotactic/chemokinetic activity. For example, a protein encoded by extended cDNAs derived from the 5' ESTs of the present invention may have chemotactic or chemokinetic activity (e.g., act as a chemokine) for mammalian cells, including, for example, monocytes, fibroblasts, neutrophils, T-cells, mast cells, eosinophils, epithelial and/or endothelial cells. Chemotactic and chemokinetic proteins can be used to mobilize or attract a desired cell population to a desired site of action. Chemotactic or chemokinetic proteins

provide particular advantages in treatment of wounds and other trauma to tissues, as well as in treatment of localized infections. For example, attraction of lymphocytes, monocytes or neutrophils to tumors or sites of infection may result in improved immune responses against the tumor or infecting agent.

5 A protein or peptide has chemotactic activity for a particular cell population if it can stimulate, directly or indirectly, the directed orientation or movement of such cell population. Preferably, the protein or peptide has the ability to directly stimulate directed movement of cells. Whether a particular protein has chemotactic activity for a population of cells can be readily determined by employing such protein or peptide in any known assay for cell
10 chemotaxis.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Assays for chemotactic activity (which will identify proteins that induce or prevent chemotaxis) consist of assays that measure the ability of a protein to induce the migration of
15 cells across a membrane as well as the ability of a protein to induce the adhesion of one cell population to another cell population. Suitable assays for movement and adhesion include, without limitation, those described in: *Current Protocols in Immunology*, Ed by Coligan, Kruisbeek, Margulies, Shevach and Strober, Pub. Greene Publishing Associates and Wiley-Interscience, Chapter 6.12: 6.12.1-6.12.28; Taub *et al.*, *J. Clin. Invest.* 95:1370-1376, 1995;
20 Lind *et al.*, *APMIS* 103:140-146, 1995; Mueller *et al.*, *Eur. J. Immunol.* 25:1744-1748; Gruber *et al.*, *J. Immunol.* 152:5860-5867, 1994; Johnston *et al.* *J. Immunol.*, 153:1762-1768, 1994.

EXAMPLE 38

Assaying the Proteins Expressed from Extended cDNAs or Portions Thereof for Regulation of Blood Clotting

25 The proteins encoded by the extended cDNAs or portions thereof may also be evaluated for their effects on blood clotting. Numerous assays for such activity are familiar to those skilled in the art, including the assays disclosed in the following references, which are
30 incorporated herein by reference: Linet *et al.*, *J. Clin. Pharmacol.* 26:131-140, 1986; Burdick

et al., *Thrombosis Res.* 45:413-419, 1987; Humphrey *et al.*, *Fibrinolysis* 5:71-79, 1991; Schaub, *Prostaglandins* 35:467-474, 1988.

Those proteins which are involved in the regulation of blood clotting may then be formulated as pharmaceuticals and used to treat clinical conditions in which regulation of blood clotting is beneficial. For example, a protein of the invention may also exhibit hemostatic or thrombolytic activity. As a result, such a protein is expected to be useful in treatment of various coagulations disorders (including hereditary disorders, such as hemophilias) or to enhance coagulation and other hemostatic events in treating wounds resulting from trauma, surgery or other causes. A protein of the invention may also be useful for dissolving or inhibiting formation of thromboses and for treatment and prevention of conditions resulting therefrom (such as infarction of cardiac and central nervous system vessels (e.g., stroke)). Alternatively, as described in more detail below, genes encoding blood clotting activity proteins or nucleic acids regulating the expression of such proteins may be introduced into appropriate host cells to increase or decrease the expression of the proteins as desired.

EXAMPLE 39

Assaying the Proteins Expressed from Extended cDNAs or Portions Thereof for Involvement in Receptor/Ligand Interactions

The proteins encoded by the extended cDNAs or a portion thereof may also be evaluated for their involvement in receptor/ligand interactions. Numerous assays for such involvement are familiar to those skilled in the art, including the assays disclosed in the following references, which are incorporated herein by reference: Chapter 7. 7.28.1-7.28.22 in *Current Protocols in Immunology*, Coligan *et al.* Eds. Greene Publishing Associates and Wiley-Interscience; Takai *et al.*, *Proc. Natl. Acad. Sci. USA* 84:6864-6868, 1987; Bierer *et al.*, *J. Exp. Med.* 168:1145-1156, 1988; Rosenstein *et al.*, *J. Exp. Med.* 169:149-160, 1989; Stoltenborg *et al.*, *J. Immunol. Methods* 175:59-68, 1994; Stitt *et al.*, *Cell* 80:661-670, 1995; Gyuris *et al.*, *Cell* 75:791-803, 1993.

For example, the proteins encoded by extended cDNAs derived from the 5' ESTs of the present invention may also demonstrate activity as receptors, receptor ligands or inhibitors or agonists of receptor/ligand interactions. Examples of such receptors and ligands include,

without limitation, cytokine receptors and their ligands, receptor kinases and their ligands, receptor phosphatases and their ligands, receptors involved in cell-cell interactions and their ligands (including without limitation, cellular adhesion molecules (such as selectins, integrins and their ligands) and receptor/ligand pairs involved in antigen presentation, antigen
5 recognition and development of cellular and humoral immune responses). Receptors and ligands are also useful for screening of potential peptide or small molecule inhibitors of the relevant receptor/ligand interaction. A protein encoded by extended cDNAs derived from the 5' ESTs of the present invention (including, without limitation, fragments of receptors and
ligands) may themselves be useful as inhibitors of receptor/ligand interactions. Alternatively,
10 as described in more detail below, genes encoding proteins involved in receptor/ligand interactions or nucleic acids regulating the expression of such proteins may be introduced into appropriate host cells to increase or decrease the expression of the proteins as desired.

EXAMPLE 40

15 Assaying the Proteins Expressed from Extended cDNAs or Portions Thereof for Anti-Inflammatory Activity

The proteins encoded by the extended cDNAs or a portion thereof may also be evaluated for anti-inflammatory activity. The anti-inflammatory activity may be achieved by
20 providing a stimulus to cells involved in the inflammatory response, by inhibiting or promoting cell-cell interactions (such as, for example, cell adhesion), by inhibiting or promoting chemotaxis of cells involved in the inflammatory process, inhibiting or promoting cell extravasation, or by stimulating or suppressing production of other factors which more directly inhibit or promote an inflammatory response. Proteins exhibiting such activities can be used to treat inflammatory conditions including chronic or acute conditions, including
25 without limitation inflammation associated with infection (such as septic shock, sepsis or systemic inflammatory response syndrome), ischemia-reperfusion injury, endotoxin lethality, arthritis, complement-mediated hyperacute rejection, nephritis, cytokine- or chemokine-induced lung injury, inflammatory bowel disease, Crohn's disease or resulting from over production of cytokines such as TNF or IL-1. Proteins of the invention may also be useful to
30 treat anaphylaxis and hypersensitivity to an antigenic substance or material. Alternatively, as described in more detail below, genes encoding anti-inflammatory activity proteins or nucleic

acids regulating the expression of such proteins may be introduced into appropriate host cells to increase or decrease the expression of the proteins as desired.

EXAMPLE 41

5 Assaying the Proteins Expressed from Extended cDNAs or
 Portions Thereof for Tumor Inhibition Activity

 The proteins encoded by the extended cDNAs or a portion thereof may also be evaluated for tumor inhibition activity. In addition to the activities described above for immunological treatment or prevention of tumors, a protein of the invention may exhibit other
10 anti-tumor activities. A protein may inhibit tumor growth directly or indirectly (such as, for example, via ADCC). A protein may exhibit its tumor inhibitory activity by acting on tumor tissue or tumor precursor tissue, by inhibiting formation of tissues necessary to support tumor growth (such as, for example, by inhibiting angiogenesis), by causing production of other factors, agents or cell types which inhibit tumor growth, or by suppressing, eliminating or
15 inhibiting factors, agents or cell types which promote tumor growth. Alternatively, as described in more detail below, genes tumor inhibition activity proteins or nucleic acids regulating the expression of such proteins may be introduced into appropriate host cells to increase or decrease the expression of the proteins as desired.

20 A protein of the invention may also exhibit one or more of the following additional activities or effects: inhibiting the growth, infection or function of, or killing, infectious agents, including, without limitation, bacteria, viruses, fungi and other parasites; effecting (suppressing or enhancing) bodily characteristics, including, without limitation, height, weight, hair color, eye color, skin, fat to lean ratio or other tissue pigmentation, or organ or
25 body part size or shape (such as, for example, breast augmentation or diminution, change in bone form or shape); effecting biorhythms or circadian cycles or rhythms; effecting the fertility of male or female subjects; effecting the metabolism, catabolism, anabolism, processing, utilization, storage or elimination of dietary fat, lipid, protein, carbohydrate, vitamins, minerals, cofactors or other nutritional factors or component(s); effecting behavioral
30 characteristics, including, without limitation, appetite, libido, stress, cognition (including cognitive disorders), depression (including depressive disorders) and violent behaviors;

providing analgesic effects or other pain reducing effects; promoting differentiation and growth of embryonic stem cells in lineages other than hematopoietic lineages; hormonal or endocrine activity; in the case of enzymes, correcting deficiencies of the enzyme and treating deficiency-related diseases; treatment of hyperproliferative disorders (such as, for example, psoriasis); immunoglobulin-like activity (such as, for example, the ability to bind antigens or complement); and the ability to act as an antigen in a vaccine composition to raise an immune response against such protein or another material or entity which is cross-reactive with such protein. Alternatively, as described in more detail below, genes encoding proteins involved in any of the above mentioned activities or nucleic acids regulating the expression of such proteins may be introduced into appropriate host cells to increase or decrease the expression of the proteins as desired.

EXAMPLE 42

Identification of Proteins which Interact with Polypeptides Encoded by Extended cDNAs

Proteins which interact with the polypeptides encoded by cDNAs derived from the 5' ESTs or fragments thereof, such as receptor proteins, may be identified using two hybrid systems such as the Matchmaker Two Hybrid System 2 (Catalog No. K1604-1, Clontech). As described in the manual accompanying the kit which is incorporated herein by reference, the the cDNAs derived from 5' ESTs, or fragments thereof, are inserted into an expression vector such that they are in frame with DNA encoding the DNA binding domain of the yeast transcriptional activator GAL4. cDNAs in a cDNA library which encode proteins which might interact with the polypeptides encoded by the extended cDNAs or portions thereof are inserted into a second expression vector such that they are in frame with DNA encoding the activation domain of GAL4. The two expression plasmids are transformed into yeast and the yeast are plated on selection medium which selects for expression of selectable markers on each of the expression vectors as well as GAL4 dependent expression of the HIS3 gene. Transformants capable of growing on medium lacking histidine are screened for GAL4 dependent lacZ expression. Those cells which are positive in both the histidine selection and the lacZ assay contain plasmids encoding proteins which interact with the polypeptide encoded by the extended cDNAs or portions thereof.

Alternatively, the system described in Lustig *et al.*, *Methods in Enzymology* 283: 83-99, 1997, and in U.S. Patent No. 5,654,150, the disclosure of which is incorporated herein by reference, may be used for identifying molecules which interact with the polypeptides encoded by extended cDNAs. In such systems, *in vitro* transcription reactions are performed on a pool of vectors containing extended cDNA inserts cloned downstream of a promoter which drives *in vitro* transcription. The resulting pools of mRNAs are introduced into *Xenopus laevis* oocytes. The oocytes are then assayed for a desired activity.

Alternatively, the pooled *in vitro* transcription products produced as described above may be translated *in vitro*. The pooled *in vitro* translation products can be assayed for a desired activity or for interaction with a known polypeptide.

Proteins or other molecules interacting with polypeptides encoded by extended cDNAs can be found by a variety of additional techniques. In one method, affinity columns containing the polypeptide encoded by the extended cDNA or a portion thereof can be constructed. In some versions, of this method the affinity column contains chimeric proteins in which the protein encoded by the extended cDNA or a portion thereof is fused to glutathione S-transferase. A mixture of cellular proteins or pool of expressed proteins as described above and is applied to the affinity column. Proteins interacting with the polypeptide attached to the column can then be isolated and analyzed on 2-D electrophoresis gel as described in Ramunsen *et al.*, *Electrophoresis* 18:588-598, 1997, the disclosure of which is incorporated herein by reference. Alternatively, the proteins retained on the affinity column can be purified by electrophoresis based methods and sequenced. The same method can be used to isolate antibodies, to screen phage display products, or to screen phage display human antibodies.

Proteins interacting with polypeptides encoded by extended cDNAs or portions thereof can also be screened by using an Optical Biosensor as described in Edwards and Leatherbarrow, *Analytical Biochemistry* 246:1-6, 1997, the disclosure of which is incorporated herein by reference. The main advantage of the method is that it allows the determination of the association rate between the protein and other interacting molecules. Thus, it is possible to specifically select interacting molecules with a high or low association rate. Typically a target molecule is linked to the sensor surface (through a carboxymethyl dextran matrix) and a sample of test molecules is placed in contact with

the target molecules. The binding of a test molecule to the target molecule causes a change in the refractive index and/ or thickness. This change is detected by the Biosensor provided it occurs in the evanescent field (which extend a few hundred nanometers from the sensor surface). In these screening assays, the target molecule can
5 be one of the polypeptides encoded by extended cDNAs or a portion thereof and the test sample can be a collection of proteins extracted from tissues or cells, a pool of expressed proteins, combinatorial peptide and/ or chemical libraries, or phage displayed peptides. The tissues or cells from which the test proteins are extracted can originate from any species.

10 In other methods, a target protein is immobilized and the test population is a collection of unique polypeptides encoded by the extended cDNAs or portions thereof.

To study the interaction of the proteins encoded by the extended cDNAs or portions thereof with drugs, the microdialysis coupled to HPLC method described by Wang *et al.*, *Chromatographia* 44:205-208, 1997 or the affinity capillary electrophoresis
15 method described by Busch *et al.*, *J. Chromatogr.* 777:311-328, 1997, the disclosures of which are incorporated herein by reference can be used.

It will be appreciated by those skilled in the art that the proteins expressed from the extended cDNAs or portions may be assayed for numerous activities in addition to those
20 specifically enumerated above. For example, the expressed proteins may be evaluated for applications involving control and regulation of inflammation, tumor proliferation or metastasis, infection, or other clinical conditions. In addition, the proteins expressed from the extended cDNAs or portions thereof may be useful as nutritional agents or cosmetic agents.

The proteins expressed from the cDNAs or portions thereof may be used to generate
25 antibodies capable of specifically binding to the expressed protein or fragments thereof as described in Example 40 below. The antibodies may capable of binding a full length protein encoded by a cDNA derived from a 5' EST, a mature protein (*i.e.* the protein generated by cleavage of the signal peptide) encoded by a cDNA derived from a 5' EST, or a signal peptide encoded by a cDNA derived from a 5' EST. Alternatively, the antibodies may be
30 capable of binding fragments of at least 10 amino acids of the proteins encoded by the above cDNAs. In some embodiments, the antibodies may be capable of binding fragments of at

least 15 amino acids of the proteins encoded by the above cDNAs. In other embodiments, the antibodies may be capable of binding fragments of at least 25 amino acids of the proteins expressed from the extended cDNAs which comprise at least 25 amino acids of the proteins encoded by the above cDNAs. In further embodiments, the antibodies may be capable of binding fragments of at least 40 amino acids of the proteins encoded by the above cDNAs.

EXAMPLE 43

Production of an Antibody to a Human Protein

Substantially pure protein or polypeptide is isolated from the transfected or transformed cells as described in Example 30. The concentration of protein in the final preparation is adjusted, for example, by concentration on an Amicon filter device, to the level of a few $\mu\text{g/ml}$. Monoclonal or polyclonal antibody to the protein can then be prepared as follows:

1. Monoclonal Antibody Production by Hybridoma Fusion

Monoclonal antibody to epitopes of any of the peptides identified and isolated as described can be prepared from murine hybridomas according to the classical method of Kohler, and Milstein, *Nature* 256:495, 1975 or derivative methods thereof. Briefly, a mouse is repetitively inoculated with a few micrograms of the selected protein or peptides derived therefrom over a period of a few weeks. The mouse is then sacrificed, and the antibody producing cells of the spleen isolated. The spleen cells are fused by means of polyethylene glycol with mouse myeloma cells, and the excess unfused cells destroyed by growth of the system on selective media comprising aminopterin (HAT media). The successfully fused cells are diluted and aliquots of the dilution placed in wells of a microtiter plate where growth of the culture is continued. Antibody-producing clones are identified by detection of antibody in the supernatant fluid of the wells by immunoassay procedures, such as ELISA, as originally described by Engvall, *Meth. Enzymol.* 70:419, 1980, the disclosure of which is incorporated herein by reference and derivative methods thereof. Selected positive clones can be expanded and their monoclonal antibody product harvested for use. Detailed procedures for monoclonal antibody production are described in Davis *et al.* in *Basic Methods in Molecular Biology*

Elsevier, New York. Section 21-2, the disclosure of which is incorporated herein by reference.

2. Polyclonal Antibody Production by Immunization

5 Polyclonal antiserum containing antibodies to heterogenous epitopes of a single protein can be prepared by immunizing suitable animals with the expressed protein or peptides derived therefrom, which can be unmodified or modified to enhance immunogenicity. Effective polyclonal antibody production is affected by many factors related both to the antigen and the host species. For example, small molecules tend to be less
10 immunogenic than others and may require the use of carriers and adjuvant. Also, host animals response vary depending on site of inoculations and doses, with both inadequate or excessive doses of antigen resulting in low titer antisera. Small doses (ng level) of antigen administered at multiple intradermal sites appears to be most reliable. An effective immunization protocol for rabbits can be found in Vaitukaitis. *et al*, *J. Clin. Endocrinol. Metab.* 33:988-991 (1971), the disclosure of which is incorporated herein by reference.

15 Booster injections can be given at regular intervals, and antiserum harvested when antibody titer thereof, as determined semi-quantitatively, for example, by double immunodiffusion in agar against known concentrations of the antigen, begins to fall. See, for example, Ouchterlony, *et al.*, Chap. 19 in: *Handbook of Experimental Immunology* D. Wier (ed) Blackwell (1973), the disclosure of which is incorporated herein by reference. Plateau
20 concentration of antibody is usually in the range of 0.1 to 0.2 mg/ml of serum (about 12 μ M). Affinity of the antisera for the antigen is determined by preparing competitive binding curves, as described, for example, by Fisher, D., Chap. 42 in: *Manual of Clinical Immunology*, 2d Ed. (Rose and Friedman, Eds.) Amer. Soc. For Microbiol., Washington, D.C. (1980), the
25 disclosure of which is incorporated herein by reference.

Antibody preparations prepared according to either protocol are useful in quantitative immunoassays which determine concentrations of antigen-bearing substances in biological samples; they are also used semi-quantitatively or qualitatively to identify the presence of antigen in a biological sample. The antibodies may also be used in
30 therapeutic compositions for killing cells expressing the protein or reducing the levels of the protein in the body.

V. Use of 5' ESTs or Sequences Obtainable Therefrom or Portions Thereof as Reagents

The 5' ESTs of the present invention (or cDNAs or genomic DNAs obtainable therefrom) may be used as reagents in isolation procedures, diagnostic assays, and forensic procedures. For example, sequences from the 5' ESTs (or cDNAs or genomic DNAs obtainable therefrom) may be detectably labeled and used as probes to isolate other sequences capable of hybridizing to them. In addition, sequences from 5' ESTs (or cDNAs or genomic DNAs obtainable therefrom) may be used to design PCR primers to be used in isolation, diagnostic, or forensic procedures.

10

1. Use of 5' ESTs or Sequences Obtainable Therefrom or Portions Thereof in Isolation, Diagnostic and Forensic Procedures

EXAMPLE 44

15

Preparation of PCR Primers and Amplification of DNA

The 5' EST sequences (or cDNAs or genomic DNAs obtainable therefrom) may be used to prepare PCR primers for a variety of applications, including isolation procedures for cloning nucleic acids capable of hybridizing to such sequences, diagnostic techniques and forensic techniques. The PCR primers are at least 10 bases, and preferably at least 12, 15, or 17 bases in length. More preferably, the PCR primers are at least 20-30 bases in length. In some embodiments, the PCR primers may be more than 30 bases in length. It is preferred that the primer pairs have approximately the same G/C ratio, so that melting temperatures are approximately the same. A variety of PCR techniques are familiar to those skilled in the art. For a review of PCR technology, see *Molecular Cloning to Genetic Engineering*, White Ed. *in Methods in Molecular Biology* 67: Humana Press, Totowa 1997, the disclosure of which is incorporated herein by reference. In each of these PCR procedures, PCR primers on either side of the nucleic acid sequences to be amplified are added to a suitably prepared nucleic acid sample along with dNTPs and a thermostable polymerase such as Taq polymerase, Pfu polymerase, or Vent polymerase. The nucleic acid in the sample is denatured and the PCR primers are specifically hybridized to complementary nucleic acid sequences in the sample. The hybridized primers are extended. Thereafter, another cycle of denaturation,

20
25
30

hybridization, and extension is initiated. The cycles are repeated multiple times to produce an amplified fragment containing the nucleic acid sequence between the primer sites.

EXAMPLE 45

Use of 5'ESTs as Probes

Probes derived from 5' ESTs (or cDNAs or genomic DNAs obtainable therefrom), including full length cDNAs or genomic sequences, may be labeled with detectable labels familiar to those skilled in the art, including radioisotopes and non-radioactive labels, to provide a detectable probe. The detectable probe may be single stranded or double stranded and may be made using techniques known in the art, including *in vitro* transcription, nick translation, or kinase reactions. A nucleic acid sample containing a sequence capable of hybridizing to the labeled probe is contacted with the labeled probe. If the nucleic acid in the sample is double stranded, it may be denatured prior to contacting the probe. In some applications, the nucleic acid sample may be immobilized on a surface such as a nitrocellulose or nylon membrane. The nucleic acid sample may comprise nucleic acids obtained from a variety of sources, including genomic DNA, cDNA libraries, RNA, or tissue samples.

Procedures used to detect the presence of nucleic acids capable of hybridizing to the detectable probe include well known techniques such as Southern blotting, Northern blotting, dot blotting, colony hybridization, and plaque hybridization. In some applications, the nucleic acid capable of hybridizing to the labeled probe may be cloned into vectors such as expression vectors, sequencing vectors, or *in vitro* transcription vectors to facilitate the characterization and expression of the hybridizing nucleic acids in the sample. For example, such techniques may be used to isolate and clone sequences in a genomic library or cDNA library which are capable of hybridizing to the detectable probe as described in Example 30 above.

PCR primers made as described in Example 44 above may be used in forensic analyses, such as the DNA fingerprinting techniques described in Examples 46-50 below. Such analyses may utilize detectable probes or primers based on the sequences of the the 5' ESTs or of cDNAs or genomic DNAs isolated using the 5' ESTs.

EXAMPLE 46**Forensic Matching by DNA Sequencing**

In one exemplary method, DNA samples are isolated from forensic specimens of, for example, hair, semen, blood or skin cells by conventional methods. A panel of PCR primers based on a number of the 5' ESTs of Example 25, or cDNAs or genomic DNAs isolated therefrom as described above, is then utilized in accordance with Example 44 to amplify DNA of approximately 100-200 bases in length from the forensic specimen. Corresponding sequences are obtained from a test subject. Each of these identification DNAs is then sequenced using standard techniques, and a simple database comparison determines the differences, if any, between the sequences from the subject and those from the sample. Statistically significant differences between the suspect's DNA sequences and those from the sample conclusively prove a lack of identity. This lack of identity can be proven, for example, with only one sequence. Identity, on the other hand, should be demonstrated with a large number of sequences, all matching. Preferably, a minimum of 50 statistically identical sequences of 100 bases in length are used to prove identity between the suspect and the sample.

EXAMPLE 47**Positive Identification by DNA Sequencing**

The technique outlined in the previous example may also be used on a larger scale to provide a unique fingerprint-type identification of any individual. In this technique, primers are prepared from a large number of 5'EST sequences from Example 25, or cDNA or genomic DNA sequences obtainable therefrom. Preferably, 20 to 50 different primers are used. These primers are used to obtain a corresponding number of PCR-generated DNA segments from the individual in question in accordance with Example 44. Each of these DNA segments is sequenced, using the methods set forth in Example 46. The database of sequences generated through this procedure uniquely identifies the individual from whom the sequences were obtained. The same panel of primers may then be used at any later time to absolutely correlate tissue or other biological specimen with that individual.

EXAMPLE 48**Southern Blot Forensic Identification**

The procedure of Example 47 is repeated to obtain a panel of at least 10 amplified sequences from an individual and a specimen. Preferably, the panel contains at least 50 amplified sequences. More preferably, the panel contains 100 amplified sequences. In some embodiments, the panel contains 200 amplified sequences. This PCR-generated DNA is then digested with one or a combination of, preferably, four base specific restriction enzymes. Such enzymes are commercially available and known to those of skill in the art. After digestion, the resultant gene fragments are size separated in multiple duplicate wells on an agarose gel and transferred to nitrocellulose using Southern blotting techniques well known to those with skill in the art. For a review of Southern blotting see Davis *et al.* (Basic Methods in Molecular Biology, 1986, Elsevier Press. pp 62-65), the disclosure of which is incorporated herein by reference..

A panel of probes based on the sequences of 5' ESTs (or cDNAs or genomic DNAs obtainable therefrom), or fragments thereof of at least 10 bases, are radioactively or colorimetrically labeled using methods known in the art, such as nick translation or end labeling, and hybridized to the Southern blot using techniques known in the art (Davis *et al.*, supra). Preferably, the probe comprises at least 12, 15, or 17 consecutive nucleotides from the 5' EST (or cDNAs or genomic DNAs obtainable therefrom). More preferably, the probe comprises at least 20-30 consecutive nucleotides from the 5' EST (or cDNAs or genomic DNAs obtainable therefrom). In some embodiments, the probe comprises more than 30 nucleotides from the 5' EST (or cDNAs or genomic DNAs obtainable therefrom).

Preferably, at least 5 to 10 of these labeled probes are used, and more preferably at least about 20 or 30 are used to provide a unique pattern. The resultant bands appearing from the hybridization of a large sample of 5' EST (or cDNAs or genomic DNAs obtainable therefrom) will be a unique identifier. Since the restriction enzyme cleavage will be different for every individual, the band pattern on the Southern blot will also be unique. Increasing the number of 5' EST (or cDNAs or genomic DNAs obtainable therefrom) probes will provide a statistically higher level of confidence in the identification since there will be an increased number of sets of bands used for identification.

EXAMPLE 49**Dot Blot Identification Procedure**

Another technique for identifying individuals using the 5' EST sequences disclosed herein utilizes a dot blot hybridization technique.

5 Genomic DNA is isolated from nuclei of subject to be identified. Oligonucleotide probes of approximately 30 bp in length are synthesized that correspond to at least 10, preferably 50 sequences from the 5' ESTs or cDNAs or genomic DNAs obtainable therefrom. The probes are used to hybridize to the genomic DNA through conditions known to those in the art. The oligonucleotides are end labeled with P³² using polynucleotide kinase
10 (Pharmacia). Dot Blots are created by spotting the genomic DNA onto nitrocellulose or the like using a vacuum dot blot manifold (BioRad, Richmond California). The nitrocellulose filter containing the genomic sequences is baked or UV linked to the filter, prehybridized and hybridized with labeled probe using techniques known in the art (Davis *et al.*, *supra*). The ³²P labeled DNA fragments are sequentially hybridized with successively stringent conditions to
15 detect minimal differences between the 30 bp sequence and the DNA. Tetramethylammonium chloride is useful for identifying clones containing small numbers of nucleotide mismatches (Wood *et al.*, *Proc. Natl. Acad. Sci. USA* **82**(6):1585-1588, 1985) which is hereby incorporated by reference. A unique pattern of dots distinguishes one individual from another individual.

20 5' EST sequences (or cDNAs or genomic DNAs obtainable therefrom) or oligonucleotides containing at least 10 consecutive bases from these sequences can be used as probes in the following alternative fingerprinting technique. Preferably, the probe comprises at least 12, 15, or 17 consecutive nucleotides from the 5' EST sequences (or cDNAs or genomic DNAs obtainable therefrom). More preferably, the probe comprises at least 20-30
25 consecutive nucleotides from the 5' EST sequences (or cDNAs or genomic DNAs obtainable therefrom). In some embodiments, the probe comprises more than 30 nucleotides from the 5' EST sequences (or cDNAs or genomic DNAs obtainable therefrom).

Preferably, a plurality of probes having sequences from different genes are used in the alternative fingerprinting technique. Example 50 below provides a representative alternative
30 fingerprinting procedure in which the probes are derived from 5'EST.

EXAMPLE 50**Alternative "Fingerprint" Identification Technique**

20-mer oligonucleotides are prepared from a large number, e.g. 50, 100, or 200, of 5'EST using commercially available oligonucleotide services such as Genset, Paris, France.

5 Cell samples from the test subject are processed for DNA using techniques well known to those with skill in the art. The nucleic acid is digested with restriction enzymes such as EcoRI and XbaI. Following digestion, samples are applied to wells for electrophoresis. The procedure, as known in the art, may be modified to accommodate polyacrylamide electrophoresis, however in this example, samples containing 5 ug of DNA are loaded into
10 wells and separated on 0.8% agarose gels. The gels are transferred onto nitrocellulose using standard Southern blotting techniques.

10 ng of each of the oligonucleotides are pooled and end-labeled with ^{32}P . The nitrocellulose is prehybridized with blocking solution and hybridized with the labeled probes. Following hybridization and washing, the nitrocellulose filter is exposed to X-Omat AR X-ray
15 film. The resulting hybridization pattern will be unique for each individual.

It is additionally contemplated within this example that the number of probe sequences used can be varied for additional accuracy or clarity.

The proteins encoded by the extended cDNAs may also be used to generate
20 antibodies as explained in Examples 30 and 43 in order to identify the tissue type or cell species from which a sample is derived as described in example 51.

EXAMPLE 51**Identification of Tissue Types or Cell Species by Means of****Labeled Tissue Specific Antibodies**

25 Identification of specific tissues is accomplished by the visualization of tissue specific antigens by means of antibody preparations according to Examples 30 and 43 which are conjugated, directly or indirectly to a detectable marker. Selected labeled antibody species bind to their specific antigen binding partner in tissue sections, cell suspensions, or in extracts
30 of soluble proteins from a tissue sample to provide a pattern for qualitative or semi-qualitative interpretation.

Antisera for these procedures must have a potency exceeding that of the native preparation, and for that reason, antibodies are concentrated to a mg/ml level by isolation of the gamma globulin fraction, for example, by ion-exchange chromatography or by ammonium sulfate fractionation. Also, to provide the most specific antisera, unwanted
5 antibodies, for example to common proteins, must be removed from the gamma globulin fraction, for example by means of insoluble immunoabsorbents, before the antibodies are labeled with the marker. Either monoclonal or heterologous antisera is suitable for either procedure.

A. Immunohistochemical techniques

10 Purified, high-titer antibodies, prepared as described above, are conjugated to a detectable marker, as described, for example, by Fudenberg, Chap. 26 in: *Basic and Clinical Immunology*, 3rd Ed. Lange, Los Altos, California, 1980, or Rose, *et al.*, Chap. 12 in: *Methods in Immunodiagnosis*, 2d Ed. John Wiley and Sons, New York (1980), the disclosures of which are incorporated herein by reference.

15 A fluorescent marker, either fluorescein or rhodamine, is preferred, but antibodies can also be labeled with an enzyme that supports a color producing reaction with a substrate, such as horseradish peroxidase. Markers can be added to tissue-bound antibody in a second step, as described below. Alternatively, the specific antitissue antibodies can be labeled with ferritin or other electron dense particles, and localization of the ferritin coupled antigen-antibody
20 complexes achieved by means of an electron microscope. In yet another approach, the antibodies are radiolabeled, with, for example ^{125}I , and detected by overlaying the antibody treated preparation with photographic emulsion.

Preparations to carry out the procedures can comprise monoclonal or polyclonal antibodies to a single protein or peptide identified as specific to a tissue type, for example,
25 brain tissue, or antibody preparations to several antigenically distinct tissue specific antigens can be used in panels, independently or in mixtures, as required.

Tissue sections and cell suspensions are prepared for immunohistochemical examination according to common histological techniques. Multiple cryostat sections (about 4 μm , unfixed) of the unknown tissue and known control, are mounted and each slide
30 covered with different dilutions of the antibody preparation. Sections of known and unknown tissues should also be treated with preparations to provide a positive control, a negative

control, for example, pre-immune sera, and a control for non-specific staining, for example, buffer.

Treated sections are incubated in a humid chamber for 30 min at room temperature, rinsed, then washed in buffer for 30-45 min. Excess fluid is blotted away, and the marker
5 developed.

If the tissue specific antibody was not labeled in the first incubation, it can be labeled at this time in a second antibody-antibody reaction, for example, by adding fluorescein- or enzyme-conjugated antibody against the immunoglobulin class of the antiserum-producing species, for example, fluorescein labeled antibody to mouse IgG. Such labeled sera are
10 commercially available.

The antigen found in the tissues by the above procedure can be quantified by measuring the intensity of color or fluorescence on the tissue section, and calibrating that signal using appropriate standards.

B. Identification of tissue specific soluble proteins

The visualization of tissue specific proteins and identification of unknown tissues from that procedure is carried out using the labeled antibody reagents and detection strategy as described for immunohistochemistry; however the sample is prepared according to an electrophoretic technique to distribute the proteins extracted from the tissue in an orderly array on the basis of molecular weight for detection.
15

A tissue sample is homogenized using a Virtis apparatus; cell suspensions are disrupted by Dounce homogenization or osmotic lysis, using detergents in either case as required to disrupt cell membranes, as is the practice in the art. Insoluble cell components such as nuclei, microsomes, and membrane fragments are removed by ultracentrifugation, and the soluble protein-containing fraction concentrated if necessary and reserved for analysis.
20

A sample of the soluble protein solution is resolved into individual protein species by conventional SDS polyacrylamide electrophoresis as described, for example, by Davis, *et al.*, Section 19-2 in: *Basic Methods in Molecular Biology*, Leder ed., Elsevier, New York, 1986, the disclosure of which is incorporated herein by reference, using a range of amounts of polyacrylamide in a set of gels to resolve the entire molecular weight range of proteins to be
25 detected in the sample. A size marker is run in parallel for purposes of estimating molecular weights of the constituent proteins. Sample size for analysis is a convenient volume of from 5
30

to 55 μ l, and containing from about 1 to 100 μ g protein. An aliquot of each of the resolved proteins is transferred by blotting to a nitrocellulose filter paper, a process that maintains the pattern of resolution. Multiple copies are prepared. The procedure, known as Western Blot Analysis, is well described in Davis, L. *et al.*, *supra* Section 19-3. One set of nitrocellulose blots is stained with Coomassie blue dye to visualize the entire set of proteins for comparison with the antibody bound proteins. The remaining nitrocellulose filters are then incubated with a solution of one or more specific antisera to tissue specific proteins prepared as described in Examples 30 and 43. In this procedure, as in procedure A above, appropriate positive and negative sample and reagent controls are run.

10 In either procedure A or B, a detectable label can be attached to the primary tissue antigen-primary antibody complex according to various strategies and permutations thereof. In a straightforward approach, the primary specific antibody can be labeled; alternatively, the unlabeled complex can be bound by a labeled secondary anti-IgG antibody. In other approaches, either the primary or secondary antibody is conjugated to a biotin molecule, which can, in a subsequent step, bind an avidin conjugated marker. According to yet another strategy, enzyme labeled or radioactive protein A, which has the property of binding to any IgG, is bound in a final step to either the primary or secondary antibody.

20 The visualization of tissue specific antigen binding at levels above those seen in control tissues to one or more tissue specific antibodies, prepared from the gene sequences identified from extended cDNA sequences, can identify tissues of unknown origin, for example, forensic samples, or differentiated tumor tissue that has metastasized to foreign bodily sites.

In addition to their applications in forensics and identification, 5' ESTs (or cDNAs or genomic DNAs obtainable therefrom) may be mapped to their chromosomal locations. Example 52 below describes radiation hybrid (RH) mapping of human chromosomal regions using 5'ESTs. Example 53 below describes a representative procedure for mapping an 5' EST to its location on a human chromosome. Example 54 below describes mapping of 5' ESTs on metaphase chromosomes by Fluorescence In Situ Hybridization (FISH). Those skilled in the art will appreciate that the method of Examples 52-54 may also be used to map cDNAs or genomic DNAs obtainable from the 5' ESTs to their chromosomal locations.

2. Use of 5' ESTs or Sequences Obtainable Therefrom or Portions Thereof in Chromosome Mapping

EXAMPLE 52

Radiation hybrid mapping of 5'ESTs to the human genome

5 Radiation hybrid (RH) mapping is a somatic cell genetic approach that can be used for high resolution mapping of the human genome. In this approach, cell lines containing one or more human chromosomes are lethally irradiated, breaking each chromosome into fragments whose size depends on the radiation dose. These fragments are rescued by fusion with cultured rodent cells, yielding subclones containing different portions of the human
10 genome. This technique is described by Benham *et al.*, *Genomics* 4:509-517, 1989; and Cox *et al.*, *Science* 250:245-250, 1990, the entire contents of which are hereby incorporated by reference. The random and independent nature of the subclones permits efficient mapping of any human genome marker. Human DNA isolated from a panel of 80-100 cell lines provides a mapping reagent for ordering 5'EST. In this approach, the frequency of breakage between
15 markers is used to measure distance, allowing construction of fine resolution maps as has been done using conventional ESTs (Schuler *et al.*, *Science* 274:540-546, 1996, hereby incorporated by reference).

 RH mapping has been used to generate a high-resolution whole genome radiation hybrid map of human chromosome 17q22-q25.3 across the genes for growth hormone (GH) and thymidine kinase (TK) (Foster *et al.*, *Genomics* 33:185-192, 1996), the region
20 surrounding the Gorlin syndrome gene (Obermayr *et al.*, *Eur. J. Hum. Genet.* 4:242-245, 1996), 60 loci covering the entire short arm of chromosome 12 (Raeymaekers *et al.*, *Genomics* 29:170-178, 1995), the region of human chromosome 22 containing the neurofibromatosis type 2 locus (Frazer *et al.*, *Genomics* 14:574-584, 1992) and 13 loci on the
25 long arm of chromosome 5 (Warrington *et al.*, *Genomics* 11:701-708, 1991).

EXAMPLE 53

Mapping of 5'ESTs to Human Chromosomes using PCR techniques

 5' ESTs (or cDNAs or genomic DNAs obtainable therefrom) may be assigned to
30 human chromosomes using PCR based methodologies. In such approaches, oligonucleotide primer pairs are designed from the 5' ESTs (or cDNAs or genomic DNAs obtainable

therefrom) to minimize the chance of amplifying through an intron. Preferably, the oligonucleotide primers are 18-23 bp in length and are designed for PCR amplification. The creation of PCR primers from known sequences is well known to those with skill in the art. For a review of PCR technology see Erlich in *PCR Technology, Principles and Applications*
5 *for DNA Amplification*, Freeman and Co., New York, 1992, the disclosure of which is incorporated herein by reference.

The primers are used in polymerase chain reactions (PCR) to amplify templates from total human genomic DNA. PCR conditions are as follows: 60 ng of genomic DNA is used as a template for PCR with 80 ng of each oligonucleotide primer, 0.6 unit of Taq polymerase,
10 and 1 μ Cu of a 32 P-labeled deoxycytidine triphosphate. The PCR is performed in a microplate thermocycler (Techne) under the following conditions: 30 cycles of 94°C, 1.4 min; 55°C, 2 min; and 72°C, 2 min; with a final extension at 72°C for 10 min. The amplified products are analyzed on a 6% polyacrylamide sequencing gel and visualized by autoradiography. If the length of the resulting PCR product is identical to the distance
15 between the ends of the primer sequences in the extended cDNA from which the primers are derived, then the PCR reaction is repeated with DNA templates from two panels of human-rodent somatic cell hybrids, BIOS PCRable DNA (BIOS Corporation) and NIGMS Human-Rodent Somatic Cell Hybrid Mapping Panel Number 1 (NIGMS, Camden, NJ).

PCR is used to screen a series of somatic cell hybrid cell lines containing defined sets
20 of human chromosomes for the presence of a given 5' EST (or cDNA or genomic DNA obtainable therefrom). DNA is isolated from the somatic hybrids and used as starting templates for PCR reactions using the primer pairs from the 5' EST (or cDNA or genomic DNA obtainable therefrom). Only those somatic cell hybrids with chromosomes containing the human gene corresponding to the 5' EST (or cDNA or genomic DNA obtainable
25 therefrom) will yield an amplified fragment. The 5' EST (or cDNA or genomic DNA obtainable therefrom) are assigned to a chromosome by analysis of the segregation pattern of PCR products from the somatic hybrid DNA templates. The single human chromosome present in all cell hybrids that give rise to an amplified fragment is the chromosome containing that 5'EST (or cDNA or genomic DNA obtainable therefrom). For a review of techniques
30 and analysis of results from somatic cell gene mapping experiments, see Ledbetter *et al.*, *Genomics* 6:475-481, 1990, the disclosure of which is incorporated herein by reference.

EXAMPLE 54

Mapping of Extended 5' ESTs to Chromosomes Using Fluorescence *In Situ*
Hybridization

Fluorescence in situ hybridization allows the 5'EST (or cDNA or genomic DNA
5 obtainable therefrom) to be mapped to a particular location on a given chromosome. The
chromosomes to be used for fluorescence in situ hybridization techniques may be obtained
from a variety of sources including cell cultures, tissues, or whole blood.

In a preferred embodiment, chromosomal localization of an 5'EST (or cDNA or
genomic DNA obtainable therefrom) is obtained by FISH as described by Cherif *et al.* (*Proc.*
10 *Natl. Acad. Sci. U.S.A.*, 87:6639-6643, 1990), the disclosure of which is incorporated herein
by reference. Metaphase chromosomes are prepared from phytohemagglutinin (PHA)-
stimulated blood cell donors. PHA-stimulated lymphocytes from healthy males are cultured
for 72 h in RPMI-1640 medium. For synchronization, methotrexate (10 μ M) is added for 17
h, followed by addition of 5-bromodeoxyuridine (5-BrdU, 0.1 mM) for 6 h. Colcemid (1
15 μ g/ml) is added for the last 15 min before harvesting the cells. Cells are collected, washed in
RPMI, incubated with a hypotonic solution of KCl (75 mM) at 37°C for 15 min and fixed in
three changes of methanol:acetic acid (3:1). The cell suspension is dropped onto a glass slide
and air dried. The 5'EST (or cDNA or genomic DNA obtainable therefrom) is labeled with
biotin-16 dUTP by nick translation according to the manufacturer's instructions (Bethesda
20 Research Laboratories, Bethesda, MD), purified using a Sephadex G-50 column (Pharmacia,
Upsala, Sweden) and precipitated. Just prior to hybridization, the DNA pellet is dissolved in
hybridization buffer (50% formamide, 2 X SSC, 10% dextran sulfate, 1 mg/ml sonicated
salmon sperm DNA, pH 7) and the probe is denatured at 70°C for 5-10 min.

Slides kept at -20°C are treated for 1 h at 37°C with RNase A (100 μ g/ml), rinsed
25 three times in 2 X SSC and dehydrated in an ethanol series. Chromosome preparations are
denatured in 70% formamide, 2 X SSC for 2 min at 70°C, then dehydrated at 4°C. The
slides are treated with proteinase K (10 μ g/100 ml in 20 mM Tris-HCl, 2 mM CaCl₂) at 37°C
for 8 min and dehydrated. The hybridization mixture containing the probe is placed on the
slide, covered with a coverslip, sealed with rubber cement and incubated overnight in a humid
30 chamber at 37°C. After hybridization and post-hybridization washes, the biotinylated probe is
detected by avidin-FITC and amplified with additional layers of biotinylated goat anti-avidin

and avidin-FITC. For chromosomal localization, fluorescent R-bands are obtained as previously described (Cherif *et al.*, *supra.*). The slides are observed under a LEICA fluorescence microscope (DMRXA). Chromosomes are counterstained with propidium iodide and the fluorescent signal of the probe appears as two symmetrical yellow-green spots
5 on both chromatids of the fluorescent R-band chromosome (red). Thus, a particular 5'EST (or cDNA or genomic DNA obtainable therefrom) may be localized to a particular cytogenetic R-band on a given chromosome.

Once the 5'EST (or cDNA or genomic DNA obtainable therefrom) have been
10 assigned to particular chromosomes using the techniques described in Examples 52-54 above, they may be utilized to construct a high resolution map of the chromosomes on which they are located or to identify the chromosomes in a sample.

EXAMPLE 55

15 Use of 5'EST to Construct or Expand Chromosome Maps

Chromosome mapping involves assigning a given unique sequence to a particular chromosome as described above. Once the unique sequence has been mapped to a given chromosome, it is ordered relative to other unique sequences located on the same chromosome. One approach to chromosome mapping utilizes a series of yeast artificial
20 chromosomes (YACs) bearing several thousand long inserts derived from the chromosomes of the organism from which the extended cDNAs (or genomic DNAs obtainable therefrom) are obtained. This approach is described in Nagaraja *et al.*, *Genome Research* 7:210-222, 1997, the disclosure of which is incorporated herein by reference. Briefly, in this approach each chromosome is broken into overlapping pieces which are inserted into the YAC vector.
25 The YAC inserts are screened using PCR or other methods to determine whether they include the 5'EST (or cDNA or genomic DNA obtainable therefrom) whose position is to be determined. Once an insert has been found which includes the 5'EST (or cDNA or genomic DNA obtainable therefrom), the insert can be analyzed by PCR or other methods to determine whether the insert also contains other sequences known to be on the chromosome
30 or in the region from which the 5'EST (or cDNA or genomic DNA obtainable therefrom) was derived. This process can be repeated for each insert in the YAC library to determine the

location of each of the extended cDNAs (or genomic DNAs obtainable therefrom) relative to one another and to other known chromosomal markers. In this way, a high resolution map of the distribution of numerous unique markers along each of the organisms chromosomes may be obtained.

5

As described in Example 56 below extended cDNAs (or genomic DNAs obtainable therefrom) may also be used to identify genes associated with a particular phenotype, such as hereditary disease or drug response.

10 3. Use of 5'ESTs or Sequences Obtained Therefrom or Fragments Thereof in Gene Identification

EXAMPLE 56

Identification of genes associated with hereditary diseases or drug response

15 This example illustrates an approach useful for the association of 5'ESTs (or cDNA or genomic DNA obtainable therefrom) with particular phenotypic characteristics. In this example, a particular 5'EST (or cDNA or genomic DNA obtainable therefrom) is used as a test probe to associate that 5'EST (or cDNA or genomic DNA obtainable therefrom) with a particular phenotypic characteristic.

20 5'ESTs (or cDNA or genomic DNA obtainable therefrom) are mapped to a particular location on a human chromosome using techniques such as those described in Examples 52 and 53 or other techniques known in the art. A search of Mendelian Inheritance in Man (McKusick in *Mendelian Inheritance in Man* (available on line through Johns Hopkins University Welch Medical Library) reveals the region of the human chromosome which contains the 5'EST (or cDNA or genomic DNA obtainable therefrom) to be a very gene rich
25 region containing several known genes and several diseases or phenotypes for which genes have not been identified. The gene corresponding to this 5'EST (or cDNA or genomic DNA obtainable therefrom) thus becomes an immediate candidate for each of these genetic diseases.

30 Cells from patients with these diseases or phenotypes are isolated and expanded in culture. PCR primers from the 5'EST (or cDNA or genomic DNA obtainable therefrom) are used to screen genomic DNA, mRNA or cDNA obtained from the

patients. 5'ESTs (or cDNA or genomic DNA obtainable therefrom) that are not amplified in the patients can be positively associated with a particular disease by further analysis. Alternatively, the PCR analysis may yield fragments of different lengths when the samples are derived from an individual having the phenotype associated with the disease than when the sample is derived from a healthy individual, indicating that the gene containing the 5'EST may be responsible for the genetic disease.

VI. Use of 5'EST (or cDNA or Genomic DNA Obtainable Therefrom) to Construct Vectors

The present 5'ESTs (or cDNA or genomic DNA obtainable therefrom) may also be used to construct secretion vectors capable of directing the secretion of the proteins encoded by genes therein. Such secretion vectors may facilitate the purification or enrichment of the proteins encoded by genes inserted therein by reducing the number of background proteins from which the desired protein must be purified or enriched. Exemplary secretion vectors are described in Example 57 below.

1. Construction of Secretion Vectors

EXAMPLE 57

Construction of Secretion Vectors

The secretion vectors include a promoter capable of directing gene expression in the host cell, tissue, or organism of interest. Such promoters include the Rous Sarcoma Virus promoter, the SV40 promoter, the human cytomegalovirus promoter, and other promoters familiar to those skilled in the art.

A signal sequence from a 5' EST (or cDNAs or genomic DNAs obtainable therefrom) is operably linked to the promoter such that the mRNA transcribed from the promoter will direct the translation of the signal peptide. The host cell, tissue, or organism may be any cell, tissue, or organism which recognizes the signal peptide encoded by the signal sequence in the 5' EST (or cDNA or genomic DNA obtainable therefrom). Suitable hosts include mammalian cells, tissues or organisms, avian cells, tissues, or organisms, insect cells, tissues or organisms, or yeast.

In addition, the secretion vector contains cloning sites for inserting genes encoding the proteins which are to be secreted. The cloning sites facilitate the cloning of the insert gene in frame with the signal sequence such that a fusion protein in which the signal peptide is fused to the protein encoded by the inserted gene is expressed from the mRNA transcribed from the promoter. The signal peptide directs the extracellular secretion of the fusion protein.

The secretion vector may be DNA or RNA and may integrate into the chromosome of the host, be stably maintained as an extrachromosomal replicon in the host, be an artificial chromosome, or be transiently present in the host. Many nucleic acid backbones suitable for use as secretion vectors are known to those skilled in the art, including retroviral vectors, SV40 vectors, Bovine Papilloma Virus vectors, yeast integrating plasmids, yeast episomal plasmids, yeast artificial chromosomes, human artificial chromosomes, P element vectors, baculovirus vectors, or bacterial plasmids capable of being transiently introduced into the host.

The secretion vector may also contain a polyA signal such that the polyA signal is located downstream of the gene inserted into the secretion vector.

After the gene encoding the protein for which secretion is desired is inserted into the secretion vector, the secretion vector is introduced into the host cell, tissue, or organism using calcium phosphate precipitation, DEAE-Dextran, electroporation, liposome-mediated transfection, viral particles or as naked DNA. The protein encoded by the inserted gene is then purified or enriched from the supernatant using conventional techniques such as ammonium sulfate precipitation, immunoprecipitation, immunochromatography, size exclusion chromatography, ion exchange chromatography, and HPLC. Alternatively, the secreted protein may be in a sufficiently enriched or pure state in the supernatant or growth media of the host to permit it to be used for its intended purpose without further enrichment.

The signal sequences may also be inserted into vectors designed for gene therapy. In such vectors, the signal sequence is operably linked to a promoter such that mRNA transcribed from the promoter encodes the signal peptide. A cloning site is located downstream of the signal sequence such that a gene encoding a protein whose secretion is desired may readily be inserted into the vector and fused to the signal sequence. The vector is introduced into an appropriate host cell. The protein expressed from the promoter is secreted extracellularly, thereby producing a therapeutic effect.

The 5' ESTs may also be used to clone sequences located upstream of the 5' ESTs which are capable of regulating gene expression, including promoter sequences, enhancer sequences, and other upstream sequences which influence transcription or translation levels. Once identified and cloned, these upstream regulatory sequences may be used in expression vectors designed to direct the expression of an inserted gene in a desired spatial, temporal, developmental, or quantitative fashion. Example 58 describes a method for cloning sequences upstream of the extended cDNAs or 5' ESTs.

2. Identification of Upstream Sequences With Promoting or Regulatory Activities

EXAMPLE 58

Use of Extended cDNAs or 5' ESTs to Clone Upstream Sequences from Genomic DNA

Sequences derived from extended cDNAs or 5' ESTs may be used to isolate the promoters of the corresponding genes using chromosome walking techniques. In one chromosome walking technique, which utilizes the GenomeWalker™ kit available from Clontech, five complete genomic DNA samples are each digested with a different restriction enzyme which has a 6 base recognition site and leaves a blunt end. Following digestion, oligonucleotide adapters are ligated to each end of the resulting genomic DNA fragments.

For each of the five genomic DNA libraries, a first PCR reaction is performed according to the manufacturer's instructions (which are incorporated herein by reference) using an outer adaptor primer provided in the kit and an outer gene specific primer. The gene specific primer should be selected to be specific for the extended cDNA or 5' EST of interest and should have a melting temperature, length, and location in the extended cDNA or 5'EST which is consistent with its use in PCR reactions. Each first PCR reaction contains 5 ng of genomic DNA, 5 µl of 10X Tth reaction buffer, 0.2 mM of each dNTP, 0.2 µM each of outer adaptor primer and outer gene specific primer, 1.1 mM of Mg(OAc)₂, and 1 µl of the Tth polymerase 50X mix in a total volume of 50 µl. The reaction cycle for the first PCR reaction is as follows: 1 min - 94°C / 2 sec - 94°C, 3 min - 72°C (7 cycles) / 2 sec - 94°C, 3 min - 67°C (32 cycles) / 5 min - 67°C.

The product of the first PCR reaction is diluted and used as a template for a second PCR reaction according to the manufacturer's instructions using a pair of nested primers which are located internally on the amplicon resulting from the first PCR

reaction. For example, 5 µl of the reaction product of the first PCR reaction mixture may be diluted 180 times. Reactions are made in a 50 µl volume having a composition identical to that of the first PCR reaction except the nested primers are used. The first nested primer is specific for the adaptor, and is provided with the GenomeWalker™ kit.

5 The second nested primer is specific for the particular extended cDNA or 5' EST for which the promoter is to be cloned and should have a melting temperature, length, and location in the extended cDNA or 5' EST which is consistent with its use in PCR reactions. The reaction parameters of the second PCR reaction are as follows: 1 min - 94°C / 2 sec - 94°C, 3 min - 72°C (6 cycles) / 2 sec - 94°C, 3 min - 67°C (25 cycles) / 5

10 min - 67°C. The product of the second PCR reaction is purified, cloned, and sequenced using standard techniques.

Alternatively, two or more human genomic DNA libraries can be constructed by using two or more restriction enzymes. The digested genomic DNA is cloned into vectors which can be converted into single stranded, circular, or linear DNA. A biotinylated

15 oligonucleotide comprising at least 15 nucleotides from the extended cDNA or 5' EST sequence is hybridized to the single stranded DNA. Hybrids between the biotinylated oligonucleotide and the single stranded DNA containing the extended cDNA or EST sequence are isolated as described in Example 29 above. Thereafter, the single stranded DNA containing the extended cDNA or EST sequence is released from the beads and

20 converted into double stranded DNA using a primer specific for the extended cDNA or 5' EST sequence or a primer corresponding to a sequence included in the cloning vector. The resulting double stranded DNA is transformed into bacteria. DNAs containing the 5' EST or extended cDNA sequences are identified by colony PCR or colony hybridization.

25 Once the upstream genomic sequences have been cloned and sequenced as described above, prospective promoters and transcription start sites within the upstream sequences may be identified by comparing the sequences upstream of the extended cDNAs or 5' ESTs with databases containing known transcription start sites, transcription factor binding sites, or promoter sequences.

30 In addition, promoters in the upstream sequences may be identified using promoter reporter vectors as described in Example .

EXAMPLE 59

Identification of Promoters in Cloned Upstream Sequences

The genomic sequences upstream of the extended cDNAs or 5' ESTs are cloned into
5 a suitable promoter reporter vector, such as the pSEAP-Basic, pSEAP-Enhancer, p β gal-
Basic, p β gal-Enhancer, or pEGFP-1 Promoter Reporter vectors available from Clontech.
Briefly, each of these promoter reporter vectors include multiple cloning sites positioned
upstream of a reporter gene encoding a readily assayable protein such as secreted alkaline
phosphatase, β galactosidase, or green fluorescent protein. The sequences upstream of the
10 extended cDNAs or 5' ESTs are inserted into the cloning sites upstream of the reporter gene
in both orientations and introduced into an appropriate host cell. The level of reporter protein
is assayed and compared to the level obtained from a vector which lacks an insert in the
cloning site. The presence of an elevated expression level in the vector containing the insert
with respect to the control vector indicates the presence of a promoter in the insert. If
15 necessary, the upstream sequences can be cloned into vectors which contain an enhancer for
augmenting transcription levels from weak promoter sequences. A significant level of
expression above that observed with the vector lacking an insert indicates that a promoter
sequence is present in the inserted upstream sequence.

Appropriate host cells for the promoter reporter vectors may be chosen based on the
20 results of the above described determination of expression patterns of the extended cDNAs
and ESTs. For example, if the expression pattern analysis indicates that the mRNA
corresponding to a particular extended cDNA or 5' EST is expressed in fibroblasts, the
promoter reporter vector may be introduced into a human fibroblast cell line.

Promoter sequences within the upstream genomic DNA may be further defined by
25 constructing nested deletions in the upstream DNA using conventional techniques such as
Exonuclease III digestion. The resulting deletion fragments can be inserted into the promoter
reporter vector to determine whether the deletion has reduced or obliterated promoter
activity. In this way, the boundaries of the promoters may be defined. If desired, potential
individual regulatory sites within the promoter may be identified using site directed
30 mutagenesis or linker scanning to obliterate potential transcription factor binding sites within
the promoter individually or in combination. The effects of these mutations on transcription

levels may be determined by inserting the mutations into the cloning sites in the promoter reporter vectors.

EXAMPLE 60

Cloning and Identification of Promoters

Using the method described in Example 58 above with 5' ESTs, sequences upstream of several genes were obtained. Using the primer pairs GGG AAG ATG GAG ATA GTA TTG CCT G (SEQ ID NO:29) and CTG CCA TGT ACA TGA TAG AGA GAT TC (SEQ ID NO:30), the promoter having the internal designation P13H2 (SEQ ID NO:31) was obtained.

Using the primer pairs GTA CCA GGGG ACT GTG ACC ATT GC (SEQ ID NO:32) and CTG TGA CCA TTG CTC CCA AGA GAG (SEQ ID NO:33), the promoter having the internal designation P15B4 (SEQ ID NO:34) was obtained.

Using the primer pairs CTG GGA TGG AAG GCA CGG TA (SEQ ID NO:35) and GAG ACC ACA CAG CTA GAC AA (SEQ ID NO:36), the promoter having the internal designation P29B6 (SEQ ID NO:37) was obtained.

Figure 4 provides a schematic description of the promoters isolated and the way they are assembled with the corresponding 5' tags. The upstream sequences were screened for the presence of motifs resembling transcription factor binding sites or known transcription start sites using the computer program MatInspector release 2.0, August 1996.

Table VII describes the transcription factor binding sites present in each of these promoters. The columns labeled matrix provides the name of the MatInspector matrix used. The column labeled position provides the 5' position of the promoter site. Numeration of the sequence starts from the transcription site as determined by matching the genomic sequence with the 5' EST sequence. The column labeled "orientation" indicates the DNA strand on which the site is found, with the + strand being the coding strand as determined by matching the genomic sequence with the sequence of the 5' EST. The column labeled "score" provides the MatInspector score found for this site. The column labeled "length" provides the length of the site in nucleotides. The column labeled "sequence" provides the sequence of the site found.

Bacterial clones containing plasmids containing the promoter sequences described above described above are presently stored in the inventor's laboratories under the internal identification numbers provided above. The inserts may be recovered from the deposited materials by growing an aliquot of the appropriate bacterial clone in the appropriate medium.

- 5 The plasmid DNA can then be isolated using plasmid isolation procedures familiar to those skilled in the art such as alkaline lysis minipreps or large scale alkaline lysis plasmid isolation procedures. If desired the plasmid DNA may be further enriched by centrifugation on a cesium chloride gradient, size exclusion chromatography, or anion exchange chromatography. The plasmid DNA obtained using these procedures may then be manipulated using standard
- 10 cloning techniques familiar to those skilled in the art. Alternatively, a PCR can be done with primers designed at both ends of the EST insertion. The PCR product which corresponds to the 5' EST can then be manipulated using standard cloning techniques familiar to those skilled in the art.

- The promoters and other regulatory sequences located upstream of the extended
- 15 cDNAs or 5' ESTs may be used to design expression vectors capable of directing the expression of an inserted gene in a desired spatial, temporal, developmental, or quantitative manner. A promoter capable of directing the desired spatial, temporal, developmental, and quantitative patterns may be selected using the results of the expression analysis described in Example 26 above. For example, if a promoter which confers a high level of expression in
- 20 muscle is desired, the promoter sequence upstream of an extended cDNA or 5' EST derived from an mRNA which is expressed at a high level in muscle, as determined by the method of Example 26, may be used in the expression vector.

- Preferably, the desired promoter is placed near multiple restriction sites to facilitate the cloning of the desired insert downstream of the promoter, such that the promoter is able
- 25 to drive expression of the inserted gene. The promoter may be inserted in conventional nucleic acid backbones designed for extrachromosomal replication, integration into the host chromosomes or transient expression. Suitable backbones for the present expression vectors include retroviral backbones, backbones from eukaryotic episomes such as SV40 or Bovine Papilloma Virus, backbones from bacterial episomes, or artificial chromosomes.

Preferably, the expression vectors also include a polyA signal downstream of the multiple restriction sites for directing the polyadenylation of mRNA transcribed from the gene inserted into the expression vector.

Following the identification of promoter sequences using the procedures of Examples
5 58-60, proteins which interact with the promoter may be identified as described in Example
61 below.

EXAMPLE 61

Identification of Proteins Which Interact with Promoter Sequences, Upstream

Regulatory Sequences, or mRNA

Sequences within the promoter region which are likely to bind transcription factors may be identified by homology to known transcription factor binding sites or through conventional mutagenesis or deletion analyses of reporter plasmids containing the promoter sequence. For example, deletions may be made in a reporter plasmid containing the promoter
15 sequence of interest operably linked to an assayable reporter gene. The reporter plasmids carrying various deletions within the promoter region are transfected into an appropriate host cell and the effects of the deletions on expression levels is assessed. Transcription factor binding sites within the regions in which deletions reduce expression levels may be further localized using site directed mutagenesis, linker scanning analysis, or other techniques familiar
20 to those skilled in the art.

Nucleic acids encoding proteins which interact with sequences in the promoter may be identified using one-hybrid systems such as those described in the manual accompanying the Matchmaker One-Hybrid System kit available from Clontech (Catalog No. K1603-1), the disclosure of which is incorporated herein by reference. Briefly, the
25 Matchmaker One-hybrid system is used as follows. The target sequence for which it is desired to identify binding proteins is cloned upstream of a selectable reporter gene and integrated into the yeast genome. Preferably, multiple copies of the target sequences are inserted into the reporter plasmid in tandem. A library comprised of fusions between cDNAs to be evaluated for the ability to bind to the promoter and the activation domain
30 of a yeast transcription factor, such as GAL4, is transformed into the yeast strain containing the integrated reporter sequence. The yeast are plated on selective media to

select cells expressing the selectable marker linked to the promoter sequence. The colonies which grow on the selective media contain genes encoding proteins which bind the target sequence. The inserts in the genes encoding the fusion proteins are further characterized by sequencing. In addition, the inserts may be inserted into expression
5 vectors or *in vitro* transcription vectors. Binding of the polypeptides encoded by the inserts to the promoter DNA may be confirmed by techniques familiar to those skilled in the art, such as gel shift analysis or DNase protection analysis.

10 VII. Use of 5' ESTs (or cDNAs or Genomic DNAs Obtainable Therefrom) in Gene Therapy

The present invention also comprises the use of 5'ESTs (or cDNA or genomic DNA obtainable therefrom) in gene therapy strategies, including antisense and triple helix strategies as described in Examples 62 and 63 below. In antisense approaches, nucleic acid sequences complementary to an mRNA are hybridized to the mRNA intracellularly, thereby blocking the
15 expression of the protein encoded by the mRNA. The antisense sequences may prevent gene expression through a variety of mechanisms. For example, the antisense sequences may inhibit the ability of ribosomes to translate the mRNA. Alternatively, the antisense sequences may block transport of the mRNA from the nucleus to the cytoplasm, thereby limiting the amount of mRNA available for translation. Another mechanism through which antisense
20 sequences may inhibit gene expression is by interfering with mRNA splicing. In yet another strategy, the antisense nucleic acid may be incorporated in a ribozyme capable of specifically cleaving the target mRNA.

EXAMPLE 62

25 Preparation and Use of Antisense Oligonucleotides

The antisense nucleic acid molecules to be used in gene therapy may be either DNA or RNA sequences. They may comprise a sequence complementary to the sequence of the 5'EST (or cDNA or genomic DNA obtainable therefrom). The antisense nucleic acids should have a length and melting temperature sufficient to permit formation of an intracellular
30 duplex with sufficient stability to inhibit the expression of the mRNA in the duplex. Strategies for designing antisense nucleic acids suitable for use in gene therapy are disclosed in Green *et*

al., *Ann. Rev. Biochem.* 55:569-597, 1986; and Izant and Weintraub, *Cell* 36:1007-1015, 1984, which are hereby incorporated by reference.

In some strategies, antisense molecules are obtained from a nucleotide sequence encoding a protein by reversing the orientation of the coding region with respect to a promoter so as to transcribe the opposite strand from that which is normally transcribed in the cell. The antisense molecules may be transcribed using *in vitro* transcription systems such as those which employ T7 or SP6 polymerase to generate the transcript. Another approach involves transcription of the antisense nucleic acids *in vivo* by operably linking DNA containing the antisense sequence to a promoter in an expression vector.

Alternatively, oligonucleotides which are complementary to the strand normally transcribed in the cell may be synthesized *in vitro*. Thus, the antisense nucleic acids are complementary to the corresponding mRNA and are capable of hybridizing to the mRNA to create a duplex. In some embodiments, the antisense sequences may contain modified sugar phosphate backbones to increase stability and make them less sensitive to RNase activity. Examples of modifications suitable for use in antisense strategies are described by Rossi *et al.*, *Pharmacol. Ther.* 50(2):245-254, 1991, which is hereby incorporated by reference.

Various types of antisense oligonucleotides complementary to the sequence of the 5'EST (or cDNA or genomic DNA obtainable therefrom) may be used. In one preferred embodiment, stable and semi-stable antisense oligonucleotides described in International Application No. PCT WO94/23026, hereby incorporated by reference, are used. In these molecules, the 3' end or both the 3' and 5' ends are engaged in intramolecular hydrogen bonding between complementary base pairs. These molecules are better able to withstand exonuclease attacks and exhibit increased stability compared to conventional antisense oligonucleotides.

In another preferred embodiment, the antisense oligodeoxynucleotides against herpes simplex virus types 1 and 2 described in International Application No. WO 95/04141, hereby incorporated by reference, are used.

In yet another preferred embodiment, the covalently cross-linked antisense oligonucleotides described in International Application No. WO 96/31523, hereby incorporated by reference, are used. These double- or single-stranded oligonucleotides comprise one or more, respectively, inter- or intra-oligonucleotide covalent cross-linkages,

wherein the linkage consists of an amide bond between a primary amine group of one strand and a carboxyl group of the other strand or of the same strand, respectively, the primary amine group being directly substituted in the 2' position of the strand nucleotide monosaccharide ring, and the carboxyl group being carried by an aliphatic spacer group substituted on a nucleotide or nucleotide analog of the other strand or the same strand,
5 respectively.

The antisense oligodeoxynucleotides and oligonucleotides disclosed in International Application No. WO 92/18522, incorporated by reference, may also be used. These molecules are stable to degradation and contain at least one transcription control recognition
10 sequence which binds to control proteins and are effective as decoys therefore. These molecules may contain "hairpin" structures, "dumbbell" structures, "modified dumbbell" structures, "cross-linked" decoy structures and "loop" structures.

In another preferred embodiment, the cyclic double-stranded oligonucleotides described in European Patent Application No. 0 572 287 A2, hereby incorporated by
15 reference are used. These ligated oligonucleotide "dumbbells" contain the binding site for a transcription factor and inhibit expression of the gene under control of the transcription factor by sequestering the factor.

Use of the closed antisense oligonucleotides disclosed in International Application No. WO 92/19732, hereby incorporated by reference, is also contemplated. Because these
20 molecules have no free ends, they are more resistant to degradation by exonucleases than are conventional oligonucleotides. These oligonucleotides may be multifunctional, interacting with several regions which are not adjacent to the target mRNA.

The appropriate level of antisense nucleic acids required to inhibit gene expression may be determined using *in vitro* expression analysis. The antisense molecule may be
25 introduced into the cells by diffusion, injection, infection, transfection or h-region-mediated import using procedures known in the art. For example, the antisense nucleic acids can be introduced into the body as a bare or naked oligonucleotide, oligonucleotide encapsulated in lipid, oligonucleotide sequence encapsidated by viral protein, or as an oligonucleotide operably linked to a promoter contained in an expression vector. The expression vector may
30 be any of a variety of expression vectors known in the art, including retroviral or viral vectors,

vectors capable of extrachromosomal replication, or integrating vectors. The vectors may be DNA or RNA.

The antisense molecules are introduced onto cell samples at a number of different concentrations preferably between $1 \times 10^{-10} \text{M}$ to $1 \times 10^{-4} \text{M}$. Once the minimum concentration that can adequately control gene expression is identified, the optimized dose is translated into a dosage suitable for use *in vivo*. For example, an inhibiting concentration in culture of 1×10^{-7} translates into a dose of approximately 0.6 mg/kg bodyweight. Levels of oligonucleotide approaching 100 mg/kg bodyweight or higher may be possible after testing the toxicity of the oligonucleotide in laboratory animals. It is additionally contemplated that cells from the vertebrate are removed, treated with the antisense oligonucleotide, and reintroduced into the vertebrate.

It is further contemplated that the antisense oligonucleotide sequence is incorporated into a ribozyme sequence to enable the antisense to specifically bind and cleave its target mRNA. For technical applications of ribozyme and antisense oligonucleotides see Rossi *et al.*, *supra*.

In a preferred application of this invention, the polypeptide encoded by the gene is first identified, so that the effectiveness of antisense inhibition on translation can be monitored using techniques that include but are not limited to antibody-mediated tests such as RIAs and ELISA, functional assays, or radiolabeling.

The 5' ESTs of the present invention (or cDNAs or genomic DNAs obtainable therefrom) may also be used in gene therapy approaches based on intracellular triple helix formation. Triple helix oligonucleotides are used to inhibit transcription from a genome. They are particularly useful for studying alterations in cell activity as it is associated with a particular gene. The 5' EST sequences (or cDNAs or genomic DNAs obtainable therefrom) of the present invention or, more preferably, a portion of those sequences, can be used to inhibit gene expression in individuals having diseases associated with expression of a particular gene. Similarly, a portion of 5' EST sequences (or cDNAs or genomic DNAs obtainable therefrom) can be used to study the effect of inhibiting transcription of a particular gene within a cell. Traditionally, homopurine sequences were considered the most useful for triple helix strategies. However, homopyrimidine sequences can also inhibit gene expression. Such homopyrimidine oligonucleotides bind to the major groove at

homopurine:homopyrimidine sequences. Thus, both types of sequences from the 5'EST or from the gene corresponding to the 5'EST are contemplated within the scope of this invention.

5

EXAMPLE 63

Preparation and Use of Triple Helix Probes

The sequences of the 5' ESTs (or cDNAs or genomic DNAs obtainable therefrom) are scanned to identify 10-mer to 20-mer homopyrimidine or homopurine stretches which could be used in triple-helix based strategies for inhibiting gene expression. Following
10 identification of candidate homopyrimidine or homopurine stretches, their efficiency in inhibiting gene expression is assessed by introducing varying amounts of oligonucleotides containing the candidate sequences into tissue culture cells which normally express the target gene. The oligonucleotides may be prepared on an oligonucleotide synthesizer or they may be purchased commercially from a company specializing in custom oligonucleotide synthesis,
15 such as GENSET, Paris, France.

The oligonucleotides may be introduced into the cells using a variety of methods known to those skilled in the art, including but not limited to calcium phosphate precipitation, DEAE-Dextran, electroporation, liposome-mediated transfection or native uptake.

Treated cells are monitored for altered cell function or reduced gene expression using
20 techniques such as Northern blotting, RNase protection assays, or PCR based strategies to monitor the transcription levels of the target gene in cells which have been treated with the oligonucleotide. The cell functions to be monitored are predicted based upon the homologies of the target gene corresponding to the extended cDNA from which the oligonucleotide was derived with known gene sequences that have been associated with a particular function. The
25 cell functions can also be predicted based on the presence of abnormal physiologies within cells derived from individuals with a particular inherited disease, particularly when the extended cDNA is associated with the disease using techniques described in Example 56.

The oligonucleotides which are effective in inhibiting gene expression in tissue culture cells may then be introduced *in vivo* using the techniques described above and in Example 62
30 at a dosage calculated based on the *in vitro* results, as described in Example 62.

In some embodiments, the natural (beta) anomers of the oligonucleotide units can be replaced with alpha anomers to render the oligonucleotide more resistant to nucleases. Further, an intercalating agent such as ethidium bromide, or the like, can be attached to the 3' end of the alpha oligonucleotide to stabilize the triple helix. For information on the generation of oligonucleotides suitable for triple helix formation see Griffin *et al.*, *Science* 245:967-971, 1989, which is hereby incorporated by this reference.

EXAMPLE 64

Use of cDNAs Obtained Using the 5' ESTs to Express an Encoded Protein in a Host Organism

The cDNAs obtained as described above using the 5' ESTs of the present invention may also be used to express an encoded protein in a host organism to produce a beneficial effect. In such procedures, the encoded protein may be transiently expressed in the host organism or stably expressed in the host organism. The encoded protein may have any of the activities described above. The encoded protein may be a protein which the host organism lacks or, alternatively, the encoded protein may augment the existing levels of the protein in the host organism.

A full length extended cDNA encoding the signal peptide and the mature protein, or an extended cDNA encoding only the mature protein is introduced into the host organism. The extended cDNA may be introduced into the host organism using a variety of techniques known to those of skill in the art. For example, the extended cDNA may be injected into the host organism as naked DNA such that the encoded protein is expressed in the host organism, thereby producing a beneficial effect.

Alternatively, the extended cDNA may be cloned into an expression vector downstream of a promoter which is active in the host organism. The expression vector may be any of the expression vectors designed for use in gene therapy, including viral or retroviral vectors. The expression vector may be directly introduced into the host organism such that the encoded protein is expressed in the host organism to produce a beneficial effect. In another approach, the expression vector may be introduced into cells *in vitro*. Cells containing the expression vector are thereafter selected and introduced into the host organism, where they express the encoded protein to produce a beneficial effect.

EXAMPLE 65

Use of Signal Peptides Encoded by 5' ESTs or Sequences obtained Therefrom
to Import Proteins Into Cells

5 The short core hydrophobic region (h) of signal peptides encoded by the 5'ESTS or extended cDNAs derived from SEQ ID NOs: 38-305 may also be used as a carrier to import a peptide or a protein of interest, so-called cargo, into tissue culture cells (Lin *et al.*, *J. Biol. Chem.*, 270: 14225-14258, 1995; Du *et al.*, *J. Peptide Res.*, 51: 235-243, 1998; Rojas *et al.*, *Nature Biotech.*, 16: 370-375, 1998).

10 When cell permeable peptides of limited size (approximately up to 25 amino acids) are to be translocated across cell membrane, chemical synthesis may be used in order to add the h region to either the C-terminus or the N-terminus to the cargo peptide of interest. Alternatively, when longer peptides or proteins are to be imported into cells, nucleic acids can be genetically engineered, using techniques familiar to those skilled in the art, in order to link the extended cDNA sequence encoding the h region to the 5' or the 3' end of a DNA
15 sequence coding for a cargo polypeptide. Such genetically engineered nucleic acids are then translated either *in vitro* or *in vivo* after transfection into appropriate cells, using conventional techniques to produce the resulting cell permeable polypeptide. Suitable hosts cells are then simply incubated with the cell permeable polypeptide which is then translocated across the membrane.

20 This method may be applied to study diverse intracellular functions and cellular processes. For instance, it has been used to probe functionally relevant domains of intracellular proteins and to examine protein-protein interactions involved in signal transduction pathways (Lin *et al.*, *supra*; Lin *et al.*, *J. Biol. Chem.*, 271: 5305-5308, 1996; Rojas *et al.*, *J. Biol. Chem.*, 271: 27456-27461, 1996; Liu *et al.*, *Proc. Natl. Acad. Sci. USA*,
25 93: 11819-11824, 1996; Rojas *et al.*, *Bioch. Biophys. Res. Commun.*, 234: 675-680, 1997).

Such techniques may be used in cellular therapy to import proteins producing therapeutic effects. For instance, cells isolated from a patient may be treated with imported therapeutic proteins and then re-introduced into the host organism.

30 Alternatively, the h region of signal peptides of the present invention could be used in combination with a nuclear localization signal to deliver nucleic acids into cell nucleus. Such oligonucleotides may be antisense oligonucleotides or oligonucleotides designed to form

triple helixes, as described in examples 62 and 63 respectively, in order to inhibit processing and/or maturation of a target cellular RNA.

As discussed above, the cDNAs or portions thereof obtained using the 5' ESTs of the present invention can be used for various purposes. The polynucleotides can be used to express recombinant protein for analysis, characterization or therapeutic use; as markers for tissues in which the corresponding protein is preferentially expressed (either constitutively or at a particular stage of tissue differentiation or development or in disease states); as molecular weight markers on Southern gels; as chromosome markers or tags (when labeled) to identify chromosomes or to map related gene positions; to compare with endogenous DNA sequences in patients to identify potential genetic disorders; as probes to hybridize and thus discover novel, related DNA sequences; as a source of information to derive PCR primers for genetic fingerprinting; for selecting and making oligomers for attachment to a "gene chip" or other support, including for examination for expression patterns; to raise anti-protein antibodies using DNA immunization techniques; and as an antigen to raise anti-DNA antibodies or elicit another immune response. Where the polynucleotide encodes a protein which binds or potentially binds to another protein (such as, for example, in a receptor-ligand interaction), the polynucleotide can also be used in interaction trap assays (such as, for example, that described in Gyuris *et al.*, *Cell* 75:791-803, 1993, the disclosure of which is hereby incorporated by reference) to identify polynucleotides encoding the other protein with which binding occurs or to identify inhibitors of the binding interaction.

The proteins or polypeptides provided by the present invention can similarly be used in assays to determine biological activity, including in a panel of multiple proteins for high-throughput screening; to raise antibodies or to elicit another immune response; as a reagent (including the labeled reagent) in assays designed to quantitatively determine levels of the protein (or its receptor) in biological fluids; as markers for tissues in which the corresponding protein is preferentially expressed (either constitutively or at a particular stage of tissue differentiation or development or in a disease state); and, of course, to isolate correlative receptors or ligands. Where the protein binds or potentially binds to another protein (such as, for example, in a receptor-ligand interaction), the protein can be used to identify the other protein with which binding occurs or to identify inhibitors of the binding interaction. Proteins

involved in these binding interactions can also be used to screen for peptide or small molecule inhibitors or agonists of the binding interaction.

Any or all of these research utilities are capable of being developed into reagent grade or kit format for commercialization as research products.

5 Methods for performing the uses listed above are well known to those skilled in the art. References disclosing such methods include without limitation *Molecular Cloning; A Laboratory Manual*, 2d ed., Cold Spring Harbor Laboratory Press, Sambrook, Fritsch and Maniatis eds., 1989, and *Methods in Enzymology; Guide to Molecular Cloning Techniques*, Academic Press, Berger and Kimmel eds., 1987.

10 Polynucleotides and proteins of the present invention can also be used as nutritional sources or supplements. Such uses include without limitation use as a protein or amino acid supplement, use as a carbon source, use as a nitrogen source and use as a source of carbohydrate. In such cases the protein or polynucleotide of the invention can be added to the feed of a particular organism or can be administered as a separate solid or liquid
15 preparation, such as in the form of powder, pills, solutions, suspensions or capsules. In the case of microorganisms, the protein or polynucleotide of the invention can be added to the medium in or on which the microorganism is cultured.

20 Although this invention has been described in terms of certain preferred embodiments, other embodiments which will be apparent to those of ordinary skill in the art in view of the disclosure herein are also within the scope of this invention. Accordingly, the scope of the invention is intended to be defined only by reference to the appended claims. All documents cited herein are incorporated herein by reference in their entirety.

Step	Search characteristic		Selection Characteristics		
	Program	Strand	Parameters	Identity (%)	Length (bp)
miscellaneous	blastn	both	S=61 X=16	90	17
tRNA	fasta	both	-	80	60
rRNA	blastn	both	S=108	80	40
mtRNA	blastn	both	S=108	80	40
Prokaryotic	blastn	both	S=144	90	40
Fungal	blastn	both	S=144	90	40
Alu	fasta*	both	-	70	40
L1	blastn	both	S=72	70	40
Repeats	blastn	both	S=72	70	40
Promoters	blastn	top	S=54 X=16	90	15†
Vertebrate	fasta*	both	S=108	90	30
ESTs	blastn	both	S=108 X=16	90	30
Proteins	blastx ^α	top	E = 0.001	-	-

Table 1: Parameters used for each step of EST analysis

* use "Quick Fast" Database scanner

† alignment further constrained to begin closer than 10bp to EST's 5' end

α using BLOSUM62 substitution matrix

TABLE II

<u>SEQ. ID NO.</u>	<u>CATEGORY</u>	<u>VON HELJNE SCORE</u>	<u>TISSUE SOURCE</u>	<u>INTERNAL DESIGNATION</u>
ID38	new	15.8	Heart	25-13-1-H10-PU
ID39	new	14	Fetal kidney	58-47-2-B11-PU
ID40	new	12.3	Dystrophic muscle	29-3-3-H8-PU
ID41	new	12.2	Fetal kidney	58-4-2-A3-PU
ID42	new	11.9	Kidney	21-10-4-G1-PU
ID43	new	11.3	Fetal kidney	58-27-3-B10-PU
ID44	new	10.7	Fetal kidney	58-35-2-F10-PU
ID45	new	10.7	Fetal kidney	58-37-2-G10-PU
ID46	new	10.6	Dystrophic muscle	29-11-1-C11-PU
ID47	new	10	Fetal kidney	58-20-4-G7-PU
ID48	new	10	Fetal kidney	58-2-4-E9-PU
ID49	new	9.6	Fetal kidney	58-37-3-D8-PU
ID50	new	9.5	Fetal kidney	58-46-1-F1-PU
ID51	new	9.2	Dystrophic muscle	29-9-4-D8-PU
ID52	new	9.2	Muscle	27-10-4-C6-PU
ID53	new	8.3	Heart	67-5-4-H9-PU
ID54	new	8.1	Fetal kidney	58-4-3-H4-PU
ID55	new	8	Muscle	27-16-3-D12-PU
ID56	new	7.9	Fetal kidney	58-54-2-C2-PU
ID57	new	7.9	Heart	25-9-3-A3-PU
ID58	new	7.9	Dystrophic muscle	29-11-3-F1-PU
ID59	new	7.9	Fetal kidney	58-32-3-G6-PU
ID60	new	7.8	Fetal kidney	58-22-2-H8-PU
ID61	new	7.8	Fetal kidney	58-2-4-H4-PU
ID62	new	7.8	Heart	67-4-3-G3-PU
ID63	new	7.8	Fetal kidney	58-24-1-G11-PU
ID64	new	7.7	Fetal kidney	58-19-3-H1-PU
ID65	new	7.5	Fetal kidney	58-45-4-B11-PU
ID66	new	7.3	Fetal kidney	58-44-2-D3-PU
ID67	new	7.2	Dystrophic muscle	29-3-3-E7-PU
ID68	new	7.1	Dystrophic muscle	29-12-3-A3-PU
ID69	new	7.1	Fetal kidney	58-14-2-B3-PU
ID70	new	7.1	Fetal kidney	58-10-3-D12-PU
ID71	new	7	Fetal kidney	58-6-2-E5-PU
ID72	new	7	Dystrophic muscle	29-7-1-C1-PU
ID73	new	6.9	Fetal kidney	58-26-4-A12-PU
ID74	new	6.9	Fetal kidney	58-7-2-H9-PU
ID75	new	6.9	Fetal kidney	58-14-2-D5-PU
ID76	new	6.7	Fetal kidney	58-3-4-E1-PU
ID77	new	6.7	Fetal kidney	58-43-4-G3-PU
ID78	new	6.7	Fetal kidney	58-11-1-G10-PU
ID79	new	6.6	Fetal kidney	58-4-4-G2-PU
ID80	new	6.6	Fetal kidney	58-41-3-D6-PU
ID81	new	6.6	Heart	25-8-2-H10-PU
ID82	new	6.5	Muscle	27-18-4-E5-PU
ID83	new	6.4	Dystrophic muscle	29-4-1-G6-PU
ID84	new	6.4	Muscle	27-10-2-B1-PU
ID85	new	6.4	Fetal kidney	58-38-1-E5-PU
ID86	new	6.3	Muscle	27-4-3-D9-PU

<u>SEQ. ID NO.</u>	<u>CATEGORY</u>	<u>VON HEIJNE SCORE</u>	<u>TISSUE SOURCE</u>	<u>INTERNAL DESIGNATION</u>
ID87	new	6.3	Fetal kidney	58-53-1-G1-PU
ID88	new	6.3	Fetal kidney	58-7-3-F6-PU
ID89	new	6.3	Heart	25-7-2-B12-PU
ID90	new	6.1	Fetal kidney	58-16-3-E11-PU
ID91	new	6	Fetal kidney	58-15-4-C2-PU
ID92	new	6	Fetal kidney	58-34-3-A9-PU
ID93	new	5.9	Fetal kidney	58-16-1-E1-PU
ID94	new	5.9	Fetal kidney	58-4-3-E6-PU
ID95	new	5.9	Fetal kidney	58-37-3-B11-PU
ID96	new	5.9	Fetal kidney	58-35-3-C6-PU
ID97	new	5.8	Fetal kidney	58-35-1-D9-PU
ID98	new	5.8	Fetal kidney	58-26-3-B2-PU
ID99	new	5.7	Fetal kidney	58-48-1-F8-PU
ID100	new	5.7	Fetal kidney	58-27-4-A6-PU
ID101	new	5.7	Fetal kidney	58-26-3-D1-PU
ID102	new	5.7	Muscle	27-19-4-B4-PU
ID103	new	5.6	Fetal kidney	58-23-3-B2-PU
ID104	new	5.5	Heart	25-1-2-C1-PU
ID105	new	5.5	Fetal kidney	58-14-3-F10-PU
ID106	new	5.5	Fetal kidney	58-25-1-E11-PU
ID107	new	5.5	Muscle	27-9-4-A10-PU
ID108	new	5.5	Heart	25-4-2-D8-PU
ID109	new	5.4	Fetal kidney	58-29-3-G8-PU
ID110	new	5.4	Fetal kidney	58-4-4-E5-PU
ID111	new	5.4	Fetal kidney	58-24-2-H2-PU
ID112	new	5.4	Muscle	27-11-2-C8-PU
ID113	new	5.4	Fetal kidney	58-41-2-E3-PU
ID114	new	5.3	Muscle	27-22-1-G8-PU
ID115	new	5.3	Dystrophic muscle	29-1-1-C9-PU
ID116	new	5.3	Fetal kidney	58-22-2-A3-PU
ID117	new	5.2	Fetal kidney	58-42-2-G1-PU
ID118	new	5.2	Fetal kidney	58-52-2-E5-PU
ID119	new	5.2	Fetal kidney	58-24-2-G2-PU
ID120	new	5.2	Fetal kidney	58-29-1-A3-PU
ID121	new	5.1	Fetal kidney	58-26-1-G8-PU
ID122	new	5.1	Fetal kidney	58-29-4-G12-PU
ID123	new	5.1	Dystrophic muscle	29-8-3-E8-PU
ID124	new	5.1	Dystrophic muscle	29-3-4-C1-PU
ID125	new	5	Fetal kidney	58-17-2-H1-PU
ID126	new	5	Fetal kidney	58-9-3-E3-PU
ID127	new	5	Muscle	27-19-3-G7-PU
ID128	new	5	Fetal kidney	58-41-3-B4-PU
ID129	new	5	Dystrophic muscle	29-7-4-G7-PU
ID130	new	5	Muscle	27-9-3-D4-PU
ID131	new	4.9	Kidney	21-3-4-C5-PU
ID132	new	4.9	Heart	25-11-2-D6-PU
ID133	new	4.9	Heart	67-7-2-F3-PU
ID134	new	4.8	Fetal kidney	58-4-3-D3-PU
ID135	new	4.8	Fetal kidney	58-49-3-B5-PU
ID136	new	4.8	Fetal kidney	58-28-3-G12-PU
ID137	new	4.7	Fetal kidney	58-53-1-A5-PU

<u>SEQ. ID NO.</u>	<u>CATEGORY</u>	<u>VON HEIJNE SCORE</u>	<u>TISSUE SOURCE</u>	<u>INTERNAL DESIGNATION</u>
ID138	new	4.7	Fetal kidney	58-3-3-E10-PU
ID139	new	4.7	Fetal kidney	58-8-1-G7-PU
ID140	new	4.6	Fetal kidney	58-23-1-G9-PU
ID141	new	4.6	Fetal kidney	58-21-1-H8-PU
ID142	new	4.6	Fetal kidney	58-54-2-E10-PU
ID143	new	4.6	Fetal kidney	58-46-3-E4-PU
ID144	new	4.6	Fetal kidney	58-6-3-G3-PU
ID145	new	4.6	Fetal kidney	58-41-2-B5-PU
ID146	new	4.6	Dystrophic muscle	29-7-3-F2-PU
ID147	new	4.5	Fetal kidney	58-2-4-G12-PU
ID148	new	4.5	Fetal kidney	58-11-2-G8-PU
ID149	new	4.4	Fetal kidney	58-17-1-C4-PU
ID150	new	4.4	Fetal kidney	58-46-1-G7-PU
ID151	new	4.4	Heart	67-3-2-F4-PU
ID152	new	4.4	Fetal kidney	58-8-4-E12-PU
ID153	new	4.4	Fetal kidney	58-4-2-D9-PU
ID154	new	4.4	Fetal kidney	58-25-1-B5-PU
ID155	new	4.4	Fetal kidney	58-15-1-C10-PU
ID156	new	4.3	Dystrophic muscle	29-4-4-A10-PU
ID157	new	4.3	Fetal kidney	58-32-3-H7-PU
ID158	new	4.3	Kidney	21-4-4-D12-PU
ID159	new	4.3	Fetal kidney	58-45-4-G9-PU
ID160	new	4.3	Fetal kidney	58-1-2-E2-PU
ID161	new	4.2	Fetal kidney	58-25-4-E6-PU
ID162	new	4.2	Fetal kidney	58-36-4-C6-PU
ID163	new	4.2	Dystrophic muscle	29-9-3-D5-PU
ID164	new	4.2	Fetal kidney	58-3-3-B8-PU
ID165	new	4.2	Heart	25-4-4-B4-PU
ID166	new	4.2	Kidney	21-10-3-A3-PU
ID167	new	4.2	Muscle	27-19-4-B5-PU
ID168	new	4.2	Fetal kidney	58-23-3-D10-PU
ID169	new	4.1	Fetal kidney	58-41-1-F8-PU
ID170	new	4.1	Heart	25-7-2-B1-PU
ID171	new	4.1	Fetal kidney	58-53-3-G4-PU
ID172	new	4.1	Fetal kidney	58-52-2-C2-PU
ID173	new	4	Muscle	27-21-4-E12-PU
ID174	new	4	Fetal kidney	58-22-2-B8-PU
ID175	new	4	Fetal kidney	58-9-3-A8-PU
ID176	new	4	Muscle	27-5-4-C10-PU
ID177	new	4	Fetal kidney	58-38-1-G5-PU
ID178	new	4	Fetal kidney	58-34-4-F6-PU
ID179	new	4	Heart	25-1-4-D2-PU
ID180	new	4	Fetal kidney	58-48-2-D6-PU
ID181	new	3.9	Fetal kidney	58-9-3-C10-PU
ID182	new	3.9	Fetal kidney	58-9-4-F2-PU
ID183	new	3.9	Fetal kidney	58-32-3-G3-PU
ID184	new	3.9	Fetal kidney	58-52-1-F6-PU
ID185	new	3.9	Fetal kidney	58-29-1-E1-PU
ID186	new	3.9	Muscle	27-3-4-A3-PU
ID187	new	3.9	Muscle	27-16-3-H2-PU
ID188	new	3.9	Fetal kidney	58-1-3-E1-PU

<u>SEQ. ID NO.</u>	<u>CATEGORY</u>	<u>VON HEIJNE SCORE</u>	<u>TISSUE SOURCE</u>	<u>INTERNAL DESIGNATION</u>
ID189	new	3.8	Kidney	21-5-4-F10-PU
ID190	new	3.8	Kidney	21-1-3-C9-PU
ID191	new	3.8	Fetal kidney	58-1-2-C7-PU
ID192	new	3.8	Fetal kidney	58-10-3-B6-PU
ID193	new	3.8	Fetal kidney	58-11-4-C8-PU
ID194	new	3.8	Heart	67-6-4-B12-PU
ID195	new	3.8	Fetal kidney	58-7-3-B5-PU
ID196	new	3.8	Fetal kidney	58-46-3-C6-PU
ID197	new	3.7	Dystrophic muscle	29-2-4-D8-PU
ID198	new	3.7	Fetal kidney	58-7-1-D10-PU
ID199	new	3.7	Kidney	21-2-4-A11-PU
ID200	new	3.6	Fetal kidney	58-45-3-B7-PU
ID201	new	3.6	Fetal kidney	58-29-1-D7-PU
ID202	new	3.6	Fetal kidney	58-16-3-B3-PU
ID203	new	3.6	Dystrophic muscle	29-7-3-C3-PU
ID204	new	3.6	Fetal kidney	58-42-3-C2-PU
ID205	new	3.5	Fetal kidney	58-38-3-G8-PU
ID206	new	3.5	Dystrophic muscle	29-6-2-B12-PU
ID207	new	3.5	Fetal kidney	58-8-1-D1-PU
ID208	new	3.5	Fetal kidney	58-24-1-H2-PU
ID209	new	3.5	Fetal kidney	58-41-4-G9-PU
ID210	ext-est-not-vrt	12.7	Muscle	27-22-3-H1-PU
ID211	ext-est-not-vrt	10.5	Fetal kidney	58-29-1-F11-PU
ID212	ext-est-not-vrt	8	Fetal kidney	58-14-2-B12-PU
ID213	ext-est-not-vrt	7.7	Fetal kidney	58-5-1-C4-PU
ID214	ext-est-not-vrt	7.1	Fetal kidney	58-37-4-C7-PU
ID215	ext-est-not-vrt	6.7	Muscle	27-21-2-C8-PU
ID216	ext-est-not-vrt	6.7	Heart	67-1-1-C8-PU
ID217	ext-est-not-vrt	6.3	Fetal kidney	58-26-3-G6-PU
ID218	ext-est-not-vrt	6.2	Fetal kidney	58-15-3-B12-PU
ID219	ext-est-not-vrt	6	Muscle	27-5-2-G11-PU
ID220	ext-est-not-vrt	6	Fetal kidney	58-8-1-H10-PU
ID221	ext-est-not-vrt	5.8	Fetal kidney	58-38-4-D2-PU
ID222	ext-est-not-vrt	5.6	Fetal kidney	58-53-2-E6-PU
ID223	ext-est-not-vrt	5.6	Fetal kidney	58-52-2-C7-PU
ID224	ext-est-not-vrt	5.5	Fetal kidney	58-34-2-E7-PU
ID225	ext-est-not-vrt	5.4	Fetal kidney	58-4-1-A2-PU
ID226	ext-est-not-vrt	5.2	Fetal kidney	58-11-1-D3-PU
ID227	ext-est-not-vrt	5.2	Fetal kidney	58-34-3-C9-PU
ID228	ext-est-not-vrt	5.2	Fetal kidney	58-35-4-H11-PU
ID229	ext-est-not-vrt	4.6	Fetal kidney	58-3-4-H7-PU
ID230	ext-est-not-vrt	4.5	Fetal kidney	58-25-1-F3-PU
ID231	ext-est-not-vrt	4.5	Fetal kidney	58-4-4-A8-PU
ID232	ext-est-not-vrt	4.4	Fetal kidney	58-11-1-C1-PU
ID233	ext-est-not-vrt	3.9	Muscle	27-19-2-F5-PU
ID234	ext-est-not-vrt	3.5	Dystrophic muscle	29-2-2-A2-PU
ID235	est-not-ext	14.1	Fetal kidney	58-29-2-B9-PU
ID236	est-not-ext	11.4	Dystrophic muscle	29-11-2-E4-PU
ID237	est-not-ext	11.2	Fetal kidney	58-7-2-A7-PU
ID238	est-not-ext	10.8	Muscle	27-22-3-G4-PU
ID239	est-not-ext	9.9	Fetal kidney	58-9-1-G1-PU

<u>SEQ. ID NO.</u>	<u>CATEGORY</u>	<u>VON HEIJNE SCORE</u>	<u>TISSUE SOURCE</u>	<u>INTERNAL DESIGNATION</u>
ID240	est-not-ext	9.7	Dystrophic muscle	29-8-1-H5-PU
ID241	est-not-ext	9.6	Fetal kidney	58-40-1-F5-PU
ID242	est-not-ext	9.5	Fetal kidney	58-6-4-G2-PU
ID243	est-not-ext	9.2	Fetal kidney	58-25-2-E7-PU
ID244	est-not-ext	8.9	Fetal kidney	58-48-1-A11-PU
ID245	est-not-ext	8.8	Fetal kidney	58-35-2-B6-PU
ID246	est-not-ext	8.5	Kidney	21-7-4-C7-PU
ID247	est-not-ext	8.4	Fetal kidney	58-45-1-E6-PU
ID248	est-not-ext	8.1	Fetal kidney	58-39-1-A12-PU
ID249	est-not-ext	8	Fetal kidney	58-46-1-C7-PU
ID250	est-not-ext	7.9	Dystrophic muscle	29-12-3-E10-PU
ID251	est-not-ext	7.9	Fetal kidney	58-17-2-D9-PU
ID252	est-not-ext	7.9	Fetal kidney	58-52-3-B7-PU
ID253	est-not-ext	7.6	Fetal kidney	58-24-3-E7-PU
ID254	est-not-ext	7.6	Heart	25-8-4-B12-PU
ID255	est-not-ext	7.6	Dystrophic muscle	29-4-4-D12-PU
ID256	est-not-ext	7.4	Muscle	27-1-2-B3-PU
ID257	est-not-ext	7.3	Fetal kidney	58-48-1-G3-PU
ID258	est-not-ext	7.3	Dystrophic muscle	29-2-3-F8-PU
ID259	est-not-ext	7.2	Fetal kidney	58-19-3-B3-PU
ID260	est-not-ext	7	Fetal kidney	58-14-2-C4-PU
ID261	est-not-ext	6.7	Fetal kidney	58-16-3-B6-PU
ID262	est-not-ext	6.6	Fetal kidney	58-9-4-F6-PU
ID263	est-not-ext	6.4	Fetal kidney	58-1-1-E3-PU
ID264	est-not-ext	6.4	Fetal kidney	58-33-3-B4-PU
ID265	est-not-ext	6.3	Dystrophic muscle	29-12-1-H1-PU
ID266	est-not-ext	6.3	Muscle	27-9-3-A5-PU
ID267	est-not-ext	6.2	Muscle	27-17-4-C12-PU
ID268	est-not-ext	6.2	Fetal kidney	58-33-1-F1-PU
ID269	est-not-ext	5.9	Fetal kidney	58-48-4-H2-PU
ID270	est-not-ext	5.9	Fetal kidney	58-42-1-A6-PU
ID271	est-not-ext	5.7	Fetal kidney	58-33-4-E1-PU
ID272	est-not-ext	5.7	Fetal kidney	58-26-2-E12-PU
ID273	est-not-ext	5.6	Fetal kidney	58-26-1-E12-PU
ID274	est-not-ext	5.5	Fetal kidney	58-54-1-D11-PU
ID275	est-not-ext	5.5	Muscle	27-9-2-F9-PU
ID276	est-not-ext	5.4	Fetal kidney	58-30-2-H10-PU
ID277	est-not-ext	5.3	Fetal kidney	58-29-1-H1-PU
ID278	est-not-ext	5.3	Kidney	21-1-4-F2-PU
ID279	est-not-ext	5.1	Fetal kidney	58-42-4-H7-PU
ID280	est-not-ext	5	Fetal kidney	58-34-3-H10-PU
ID281	est-not-ext	5	Kidney	21-7-3-B4-PU
ID282	est-not-ext	4.9	Fetal kidney	58-4-2-D12-PU
ID283	est-not-ext	4.8	Fetal kidney	58-31-2-C10-PU
ID284	est-not-ext	4.7	Fetal kidney	58-37-3-C10-PU
ID285	est-not-ext	4.7	Fetal kidney	58-1-1-D11-PU
ID286	est-not-ext	4.6	Fetal kidney	58-52-1-A11-PU
ID287	est-not-ext	4.3	Fetal kidney	58-4-3-E10-PU
ID288	est-not-ext	4.3	Heart	67-6-4-F2-PU
ID289	est-not-ext	4.2	Fetal kidney	58-49-3-G10-PU
ID290	est-not-ext	4.1	Dystrophic muscle	29-10-3-B11-PU

<u>SEQ. ID NO.</u>	<u>CATEGORY</u>	<u>VON HEIJNE SCORE</u>	<u>TISSUE SOURCE</u>	<u>INTERNAL DESIGNATION</u>
ID291	est-not-ext	4.1	Heart	25-5-4-A7-PU
ID292	est-not-ext	4.1	Fetal kidney	58-33-2-C6-PU
ID293	est-not-ext	4	Heart	25-7-3-D4-PU
ID294	est-not-ext	3.9	Heart	67-1-3-B11-PU
ID295	est-not-ext	3.9	Fetal kidney	58-23-1-G5-PU
ID296	est-not-ext	3.7	Fetal kidney	58-6-1-B6-PU
ID297	est-not-ext	3.7	Dystrophic muscle	29-6-2-H8-PU
ID298	est-not-ext	3.7	Fetal kidney	58-43-4-B8-PU
ID299	est-not-ext	3.6	Muscle	27-3-4-G9-PU
ID300	est-not-ext	3.6	Fetal kidney	58-38-1-F10-PU
ID301	est-not-ext	3.5	Heart	67-6-4-E7-PU
ID302	est-not-ext	3.5	Fetal kidney	58-54-1-E6-PU
ID303	est-not-ext	3.5	Heart	67-4-4-G7-PU
ID304	est-not-ext	3.5	Fetal kidney	58-23-4-F4-PU
ID305	ext-vrt-not-genomic	10.5	Fetal kidney	58-42-3-A12-PU

TABLE III

<u>SEQ. ID</u> <u>NO.</u>	<u>SIGNAL PEPTIDE</u>
ID38	MMWRPSVLLLLLLLLLRHGAQG
ID39	MERPLCSHLCSCLAMLALLSPLSLA
ID40	MIHLGHILFLLLLPVAAA
ID41	MAVKLGTLALLALGLAQPASA
ID42	METLGALLVLEFLLSPVEA
ID43	MLLPLLLSSLLGGSQA
ID44	MLWLLFFLVTAIHA
ID45	MAGSPSRAAGRRLQLPLLCLFLQGATA
ID46	MKWPWTCLAILCPGPVLSPPCSGPXLALALLVLPLLWP
ID47	MPSWIGAVILPLLGLLLSLPAGA
ID48	MLLHWVRSQXXSDXKLWLSLLVPSC LCA
ID49	MKYLRHRRPNATLILAIGAFTLLLFSLLVSPPTC
ID50	MPGPRVWGKYLWRSPHSGCPGAMWWLLWGV LQA
ID51	MCGPAMFPAGPPWPRVRVQVLWALLAVLLASWRLWA
ID52	MHRRKLPLTNKRQLQKXLSKFIFDELFRNILFSLRTL RMILSLLLLSTALNILA
ID53	MKLWVSALLMAWFGVLS
ID54	MQLPLALCLVCLLVHTAFR
ID55	MLCIHXXRIIQDSFIALKILLCSVAVXLSPS
ID56	MGGFFPPTREVVCANQGAAHNRDRLPFLSLFWPWAPG
ID57	MKLFYNQLVSETKHDF AHLWLLLF SFCWM
ID58	MPSESPLLFFHILFHSCFS
ID59	MSSMWSEYTIGGVKIYFPYKAYPSQLAMMNSILRGLNSKQHCLLESPTGSGKSLALLCSA LAWQQSL
ID60	MALFLELFLNSYSLLFVRFLGFVSCLQS
ID61	MNEDEKEMKEILMAGSSLSAGVSG
ID62	MGSFLLGGIPLIXLSLCLC
ID63	MLQVATTNYLELAREVKPVCLLCSGCSCAWS
ID64	MFCLAPFFLALCFPKSTS
ID65	MSESRFQPQNQGGS LQLPLQCLLCISPPVFC
ID66	MPKHCHSFITSSCLLGLLHLSSQ
ID67	MCLLFXFIXFPFLPFSFS
ID68	MASERXPNRPXCLLVASGXAEVSA
ID69	MFPDYKLGGSYLLAFQLVFLRATSG
ID70	MRRISLTSSPVRLLLXLXLLIALE
ID71	MTFLLLLFXNAGRS
ID72	MRTVVLTMKASVIEMFLVLLVTGVHS
ID73	MSSPLLVEQSSTKSPKSWWSFLAFSCISLLFIFFSIANS
ID74	MYLFCFLFSVSKTIPLLLFFHLSFL
ID75	MIVCLLILKFLSPAET
ID76	MDKSIKSSIIWSLILCFLFILHTHT
ID77	MFFIFINGFTLLLMTLAMKPRHPIFDLLLLLXXSNQ
ID78	MCPSLEEAPSVKGTLP CSGQQPFPFGASNIPLLLGRSRKVARGAPVLWPFLTWINPALS
ID79	MLQDLLSALWFCHPCCL
ID80	MMDLRPLLSLAAYLSGPHQ
ID81	MEMPPCLLPGLPLVRTSFS
ID82	MTVELWLRLRGKGLAMLHVTRGVXG
ID83	MSIEDFVNRSILLILLCSSPPDRV
ID84	MRIHYLLFALLFLFLVPVPG
ID85	MCLLTALVTQVIS

<u>SEQ. ID</u> <u>NO.</u>	<u>SIGNAL PEPTIDE</u>
ID86	MMGNPGLALVAGTPPSRS
ID87	MNHLMPLTVLHSVLEMLRTPRTPPWPCVSLLWAPRXFA
ID88	MGHVVFQDIKNSLLXLRASQLSEG
ID89	MAGGRRDYSQLFGRGPGRLSRARASVVRWSPRATACPAPPSLPDLKRQELVSRIECGRG PVGATADFFLSLLXSVSETPG
ID90	MFWXGSLWCFHSFISFSL
ID91	MAWPNVFQXGSLLSQFXXHHVVVFLLTFFSYSLHA
ID92	MILRNLWILAVGLSLPSS
ID93	MLTVNDVRFYRNVRNHFVRLCGLLHLWLKVFS
ID94	MNLKPGLPCNLFNLCLAXPFS
ID95	MMQGEAHPASALIDRTIKMRKETEARVVLAWGLLNVSMA
ID96	MMNQTHPXXLLLAHITQS
ID97	MGLPERRGLVLLSLAEILF
ID98	MWGLEEDRSYQGLRPLCWALLYNCFSS
ID99	MLCRDGSACVPRSRRLPLPAAVRAHGPMAADXXDSARGCVVFEDVFVYFSREEWELL DDAQRLLYHDMLENFALLASLGIAFSRS
ID100	MLITRLQSGIDFAIQLESTDIGSCTTLLVYVRYAWQDDFLEDFLCFLNLTSHLSG
ID101	MESQQLHCILNSNVACSFAVGAGFLAFLSCLAFLVLD
ID102	MSNKYIKPSMSPGNTDHLFLLFPRSCSS
ID103	MVELKQLGPRSFFFFLFLPPXPP
ID104	MPYVTIPYIIVYSLILPALFFFPLHC
ID105	MPPLAAVMGSLPLLCLMDLPHSVLS
ID106	MLQIPERREFLFLGFPSNSWP
ID107	MFFVHFLITLFCVVVVG
ID108	MACFGEKRHAKSCLLHLRCLQLYWA
ID109	MVDRDENILLKQIYSPLSLALQSSCCLC
ID110	MKVKPPFVSVSLCVCDCVRG
ID111	MISSCGVKYLFSHASLFFMVGSTGSLLLTSCFYTLVSS
ID112	MGGGIAESFLCNFLVSLSL
ID113	MDALERGSLRNEQALVTYAGLAYFLCCQGVIFG
ID114	MEYLFQQPGHSRGEARAAAASLETSSLWFLPLPTHVYT
ID115	MVSSMLITLSFIFA
ID116	MPLFTMNLVSALASSAXG
ID117	MICKHYCIKKNLDYLNRMVYSAQLKLILLHCSIRVFF
ID118	MKIPVWHKTCFLKSEFSFDNLSVSLPCRPSQVPSQGGKSFLLLQLIHEDKA
ID119	MGAAVFFGCTFVAFXPFA
ID120	MVGGLDPPGRRRFQKGFDWRNLWSSCWLA PLADG
ID121	MSKMPVFASLLVVSCFYQISG
ID122	MXVTQLLPFSSPDSA
ID123	MGKAWQEMRVEWGADKGNVRSSFHFLPWALGAMA
ID124	MKVMRKRKKKDQCLPGICRSLKRRKSPRSPGMKVIRLSQFLLKCWP
ID125	MTFSFFCFPPGFKPLLHXYFLXFSIXTLLWGLNC
ID126	MAGGMKVAVSPAVGPGPWGSGVGGGGTVRLLLLILSGCLVYG
ID127	MVEMTGWWQCQAEAVKGLPPLLSCSCPPPLG
ID128	MQITPGSAAGLLPLLGNAPG
ID129	MILSTWLLLTQNSVFT
ID130	MAFHSYWGKSLQSFKTFMRVCIVLALCHTSRP
ID131	MKLRFTLLPLVLHSQS
ID132	MMILGFAFCPGHFRFNIPFLVIYSFVLS
ID133	MNRVPADSPNMCLICLLSYIALGAIHA
ID134	MDLFLNLPLVIGTIP

SEQ. ID NO.	SIGNAL PEPTIDE
ID135	MXKNHRNKKSIHFPLCTIPSMXKSCTLPQRTWDXXPSFVHWXQARLQSPPXSHLVXLS VIRSTLVLSQCLC
ID136	MSFIALVYSSLSFQ
ID137	MVFDTLKSRIVLFLNSXFPIIC
ID138	MLEMEMTWRLCDECSRWGMASAWGRGGKLLGAQVALHPRNCSKAKIFLFSILLMSLRT
ID139	MDDLMLFFLGALCRESG
ID140	MVLGALNLPSQELPTLLLLPVGAPG
ID141	MLVSKIQTFFVSFLSIPVLG
ID142	MCNPVAHTFRGVHEHHAMLLSTGLNILGTQA
ID143	MQCWILLWEACTGRCQA
ID144	MTGYPWANSITTVLCILGCHGNLCC
ID145	MVSCDVXSYVIIFTALFLXLHVA
ID146	MKSFDKKLFAIFLMCLKSIG
ID147	MFGAGDEDDTDFLSPSGGARLASLFGLDQXAXG
ID148	MVLTGLESWPVLVGRRFLSLSAADGXDX
ID149	MVIELTSVFQAMIWSQG
ID150	MESTLGAGIVIAEALQNQLAWLENVWLWXXLXXXIPXILFLFYFPAAYYA
ID151	MIIVSELGTPGTGLVGVLSTFLYC
ID152	MNWNVRGTRGFLLCPLVCGLRR
ID153	MLRCGGRGLLLGLAVAAAA
ID154	MILLMIVFSIFLL
ID155	MSLLFIFRSILISC
ID156	MPLISKVLIQLSQAFWA
ID157	MDTSSVGGLELTDQTPVLLGSTAMATSLT
ID158	MDTGESFSPHTSCRGHWRILLTHVPPWILE
ID159	MPYLDPYITQPIIQIERKLVLSSVLKEPVS
ID160	MDTSSVGGLELTDQTPVLLGSTAMATSLT
ID161	MHVLFNIVTTNXXNHFGLLDFVVQCCDS
ID162	MPPQSCCSKTAYWLSFMSWAQS
ID163	MSCVFFHFLQGGLG
ID164	MSISLSSLILLPIWINMAQI
ID165	MTALNLVAPFSDGDSGSVSLASFCAVVLSPVFQ
ID166	MWSRPVQVLGGLATCQH
ID167	MRYRLRIQITTSLNQILLFLLISC
ID168	MPFFSNQPTQVSLLFFCCSPLYSP
ID169	MRVKDPTKALPEKAKRSKRPTVPHDEDSSDDIAVGLTCQHVSHA
ID170	MVSLGYLIFVLYLWLCFMQISEEKLIEHTGTYLTSSSPLCQL
ID171	MSLTSRXXIMXTIKIQNISITKVLCCLLIATPTFF
ID172	MXAEAAGVVSTSVAAAAVA
ID173	MWIMSSCLALTYTNS
ID174	MPRGVYNSNALVLVTRGSSS
ID175	MIEPCEKMKHYDMNWFLCMYECFFFHLLTEFLLPCVHPFSVIA
ID176	MAMWNRPCQXLPPQPLVAEPTAEGEPHLPTEANRFAYAAALCGISLSQXFP
ID177	MEQVCLLVSYAVDSAAG
ID178	MRKISHCLHCWPESGATLRCWASTPVSG
ID179	MCINDHIKLLHPCGSITLTSS
ID180	MRCRVALQCGLTIPALX
ID181	MTVRYGKFLSLLKDGAENDLTWVLKHCFERFLKQQQTSIKSSLLCLQGNYAGHDWVVSLLF MIMLGDKKEKTFQFLHQFSRLLTSAFLWLPRLHI
ID182	MAFDVSCFFWVVLFSAGCKV
ID183	MLTRLVLSAHLSTTSPPWTHA

SEQ. ID NO.	SIGNAL PEPTIDE
ID184	MRYFQGSPSPYSEIEIELCDHVYSFQGLCVNLLGFEPVIS
ID185	MXXKRTHXXXSVFNGLVYAAGGRNAEGSLASLECYVPSTNQ
ID186	MFLKVQSQSIFYXPYRDCLNFHKSTYLLFFHLLLNDFFT
ID187	MQPLKIIFYLSVSIWILLIYTFQCNS
ID188	MMRTTARVAACATAAPLQA
ID189	MEAATTLHPGPRPALPLGARARWASSCLHPSARS
ID190	MQGVRGPVSFSWSTTMLCPVIFFPSNCWK
ID191	MXXFSFXLLFXXFXFRQ
ID192	MLLLSEALSESVRLLFRFSVIMA
ID193	MALISLPCTTAFPLLSS
ID194	MSEEEAAQIPRSSVWEQDQQNVVQRVVALPLVRATCT
ID195	MAAAAAAGAAAGLPGPVAQGLKEALVDTLTGILSPVQEVRAAAEEQIKVLEVTEEFVHVL AELTVDPQGALA
ID196	MNSGGGFGLGLGFGLTPTSVIQVTNLSSAVTSEQMRTLFSFLGEIEELRLYPPDNAPLAF SSXVCYVKFRDPSSVGVAQHLTNTVFIDRLXSCSLCRRLVSRFXXXLYNFCPVCYC
ID197	MIEMLIPLDCVLS
ID198	MHPFLAAHGPAFHKGYKHSTINIVDIYPMCHILGLKPHPNNGTFGHTKCLLVDQWCINL PEAIAIVIGSLLVLTMLTC
ID199	MIWPMSASVATLWS
ID200	MGIDIFYPSHIPDFHPIHLFIYLVFVECLLC
ID201	MKELNQKLTNKNKIEDLEQEIQKQKQETLQEEITSLQSSVQEYEEKNXKIKQLLVKT KKELADSKQAETDHLILQASLKGELEA
ID202	MGNTLKEMQDVQGALQCYTRAIQINPAFADAHSNLASIHKDSGNIPEAIASYRTALKLKP DFPDAYCNLAHCLQIVCDWTDYDERMKKLVSIVADQLEKNRLLLILIVCYI MLILADTRRVQGGTLGLIPAVLNRVHVAYAIPIPSLFC
ID203	MLVGIYFCVFLFPLISNTSS
ID204	MFLAPSLITKLLTGSESPDGNPPALGRPLLQGACPLIFL
ID205	MDPSASKSCLFYLVQKVS
ID206	MSLTASGPRAAWEERVGGHTWGANIPTAPDSQRWLCLQAYLASFS
ID207	MKYQMVSGSAQLASPLPGATP
ID208	MNGTFPGTYVYLVAYGDLRIFGCFWGLMYXWLLG
ID209	MGPSTPLLILFLLSWSGPLQG
ID210	MKFISTSLLLMLLVSSLSPVQG
ID211	MNYQYGFNMVMSHPHAVNEIALSLNNKNPRTKALVLELLAAVCLVRG
ID212	MAQSIHMYAARVQWGLVMCFLSYFGTFA
ID213	MGSYSHSLHLFHLIRPXQG
ID214	MARCFSLVLLTISIWT
ID215	MAMRYNRLTVLAGAMLALGLMTCLSVLFGYATS
ID216	MPQQPVEQGSPLLRQLLLPLPPFSFP
ID217	MPSRSPFTWSHLCWRAGRCPRWRACLSSSVRMCSAPAAPSRFGALGXSARRWPRRDA
ID218	DTWCAPQGVMRASLLPMLLGSWA
ID219	MSHTEVKLKIPFGNKLLDAVCLVPNKSLTYGILTHGASG
ID220	MELGSCLEGGREAAEEEGEPEVKRRLLCXEFXSVASDA
ID221	MGRTYIVEETVGQYLSNINLQGAFAVSGLLIGQCSS
ID222	MGSRKCGGCLSCLLIPLALWS
ID223	MGSRKCGGCLSCLLIPLALWS
ID224	MWWFQQGLSFLPSALVIWTS
ID225	MFNASTFTDWSSSIFVFTFKSKKSAGLPLIFSLWCSGVLL
ID226	MKMASSLAFLLLNFHVSLLLQLLTPCSA
ID227	MHILQLLTTVDGIIAIVHCPDTGKDIWNLLFDLVCHFCQS
ID228	MSDQIKFIMDSLNEKPFKKNYNLITFXSLEPMQLLQVLSVLA

<u>SEQ. ID</u> <u>NO.</u>	<u>SIGNAL PEPTIDE</u>
ID229	MATSSQXRQLLSDYGPPSLGYTQGTGNSQXPQSKYAELLAILXELGKEIRPMYAGSKSAM ERLKRGIHAXGLVRECLA
ID230	MRLLGAAAVAALGRG
ID231	MAQRLLLRFLASVIS
ID232	MFRLNSLSALAEAVG
ID233	MSGSNKSHNKARTSPYPGSKVERSQVPNEKVGWLVEWQDYKPVEYTA VSVLA GPRWA
ID234	MRTTLMFSLTAQWXTS
ID235	MSDLLLLGLIGGLTLLLLLTLLAFA
ID236	MEGTEMGARPGGHPXKWSFLWSLALWLPLALS
ID237	MXFLRKVXSILSLQVLLTTVTSTVFLYFESVRTFVXESPALILLFALGSLGLIFA
ID238	MAATLGPLGSWQQWRRCLSARDGSRMLLLLLLLGSGQG
ID239	MSSWMYLGYPVTSNTTCLKLISSFPQILPFLFPFPVNA
ID240	MAPGVIIIQLCLLLPSCSL
ID241	MRHGFIQQQFSLTAFSXXXIFTLXXLSQLLSSAAPKHTAAPTALPCLQGQQLNSLSLGT SELSCVLASSCLSTKTDPSGLSLSLGASAPVQC
ID242	MFQNIQKCLNVPFVRGYHVFIYINLNAVILIFLSFLPFINS
ID243	MSLSQRGFPVLALFLSGSLA
ID244	MAARWRFWCVSVTMVMVALLIVCDVPSASA
ID245	MFAPA VMRAFRKNKTLGYGVPMLLLIVGGSFG
ID246	MELPSGPGPERLFDHRLPGDCFLLLVLLLYAPVGFC
ID247	MAQSQGWVXRYXKAFCKGFFVAVPVAVTFLDRVACVARVEGASMQPSLNPGGXSXS DVVXXNHWKVRNFEVHRGDIVSLVLLTVTPSXRQ
ID248	MSSAAADHWAWLLVLSFVFGCNV
ID249	MNLFKTNHVFFLLLLAHIIA
ID250	MPALLPVASRLLLLPRVLLTMASG
ID251	MIGSGLAGSGGAGGPSSTVTWCALFSNHVAATQASLLLSFVWMPALLPVASRLLLLPRVL LTMASG
ID252	MPALLPVASRLLLLPRVLLTMASG
ID253	MEASWGSFNAERGWYVSVQQPEEAEAEELSPLLSNELHRQRSPGVSFGLSVFNLMNAIMG SGILGLAYVMANTGVFGFSFLLLTVALLASYS
ID254	MPSSFFLLLRFFLRIDG
ID255	MKRTHLFIVGIYFLSSCRA
ID256	MGDKIWLPFPVLLAALPPVLLP
ID257	MPHSSLHPSIPCPRGHGAQKAALVLLSACLVTWGLG
ID258	MGAWGRGWPEERQGHLLLLLLPAPTLK
ID259	MGQCGITSSKTVLVFLNLIFWGAAGILCYVGAYVFITYDDYDHFFEDVYTLIPAVVIIAV RALLFIIGLIGCCAT
ID260	MPXAFSVSSFPVSIPAVLTQTDWTEPWLMGLATFHALCVLLTCLSSRSYRLQIGHFLCLV ILVYC
ID261	MLLLSLFFPLRISL
ID262	METGERARLILVLQLLLRIRR
ID263	MCGXXFSLPCLRLFLVVTCTYXLLLLHKEILGCSSVCQLCTG
ID264	MNPVTESPSCLFSPPSESALASQLALSASCDQRAPFSLAGVXSXXPRLASRQVAPPFGR ACCFLSAFSPTLT
ID265	MSRSSKVVLGLSVLLTAATVA
ID266	MGIQTSPVLLASLGVLVTLLGLAVG
ID267	MYPHYLLIXPPIPSQFLKQCPPTLSDPFLPLALRSLDVLLSSAXLVXXS
ID268	MEQKHXRELEQLKLTENKILLXTFQTWCLR
ID269	MMTAPVLAAQTLKFLTLLQKSNA
ID270	MDSAACAAAATPVPALALAXAPDLAQA

<u>SEQ. ID</u> <u>NO.</u>	<u>SIGNAL PEPTIDE</u>
ID271	MASLGLQLVGYILGLLGLLGTLVA
ID272	MASLGLQLVGYILGLLGLLGTLVA
ID273	MLCSLLLCECLLLVAGYA
ID274	MASRLCGGALWYVCPSPGAWM
ID275	MTSALTQGLERIPDQLGYLVLSEGAULA
ID276	MASPSRRLQTKPVITCFKSVLLIXTXIXWITGVILLAVGIWG
ID277	MADAASQVLLGSGLTLSQP
ID278	MSRNLRTALIFGGFISLIGA
ID279	MPHGLWCFHLVVLSTYS
ID280	MSLVAVFLSCGLIS
ID281	MMKRAAAAAVGGALAVGAVPVVLS
ID282	MAVIVDKPWFYDMKKVWEGYPIQSTIPSQYWYMIELSFYWSLLFSIASDVKRKDFKEQI IHHVATILISFSWFANYIRA
ID283	MIISLFIYIFLTCSNT
ID284	MAAELVEAKNMVMSFRVSDLQMLLGFVGRSKS
ID285	MTGLSMXGGGSXXGDVXPXYGKXGPLRXLPSPSGPLPPSSGLSQPQVHALCPLSPLVTT
ID286	MQMYSRQLASXEWLTIQGGLLGXGLXXXSLT
ID287	MASLEVSRSRRSRRELEVRSPRQNKYSVLLPTYNERENPLIVWLLVKSFSSES
ID288	MDKDSQGLLDSSLMASGTAS
ID289	MGLLTFGYIEXXKTEHNPDDHSCLAWSWEAAGCHG
ID290	MGLYAAVAGVLAGVES
ID291	MGLYAAAAGVLAGVESRQGSIKGLVYSSNFQNVKQLYALVCETQRYSAVLDAVIASA GLLRA
ID292	MGAQHTALLNTEVRWLSRGKVLVRLFELRRELLVFMDSAFRLSDCLTNSSWLLRLAYLA DIFT
ID293	MSLRNLWRDYKVLVVMVPLVGLIHL
ID294	MVLRSLVEYSQDVLAHPVSEEHLDPVSLIGEFSDPAELGKLLQLVLGCAIS
ID295	MIHGFCLAPTTSA
ID296	MXCPRTWCLACVEASPG
ID297	MADVEDGEETCALASHSGSSG
ID298	MFKVAAPPMLIXXIIMFLLIIVCGSP
ID299	MDFWDPVAFXMCLWSLRNLFS
ID300	MSPAGKHNSKFTFFVALDGSVPLLSLSHSIGI
ID301	MHWALVCVGLHTEGPWG
ID302	MFGAAARSADLVLEKNLQAAHGYAQEDRERMHRXIVSLXQNLLNFMIGSILDLWQCF LWFIYIGSSSLNGTRG
ID303	MAARWRFWCVSVTMVVALLIVCDVPSA
ID304	MVVLLLQPSMIQEVWT
ID305	MLHLHXSCLCFRSWLPAMLAVLLSLAPSASS

Minimum signal peptide score	false positive rate	false negative rate	proba(0.1)	proba(0.2)
3.5	0.121	0.036	0.467	0.664
4	0.096	0.06	0.519	0.708
4.5	0.078	0.079	0.565	0.745
5	0.062	0.098	0.615	0.782
5.5	0.05	0.127	0.659	0.813
6	0.04	0.163	0.694	0.836
6.5	0.033	0.202	0.725	0.855
7	0.025	0.248	0.763	0.878
7.5	0.021	0.304	0.78	0.889
8	0.015	0.368	0.816	0.909
8.5	0.012	0.418	0.836	0.92
9	0.009	0.512	0.856	0.93
9.5	0.007	0.581	0.863	0.934
10	0.006	0.679	0.835	0.919

TABLE IV

Minimum signal peptide score	All ESTs	New ESTs	ESTs matching public EST closer than 40 bp from beginning	ESTs extending known mRNA more than 40 bp	ESTs extending public EST more than 40 bp
3.5	2674	947	599	23	150
4	2278	784	499	23	126
4.5	1943	647	425	22	112
5	1657	523	353	21	96
5.5	1417	419	307	19	80
6	1190	340	238	18	68
6.5	1035	280	186	18	60
7	893	219	161	15	48
7.5	753	173	132	12	38
8	636	133	101	11	29
8.5	543	104	83	8	26
9	456	81	63	6	24
9.5	364	57	48	6	18
10	303	47	35	6	15

TABLE V

Tissue	All ESTs	New ESTs	ESTs matching public EST closer than 40 bp from beginning	ESTs extending known mRNA more than 40 bp	ESTs extending public EST more than 40 bp
Brain	329	131	75	3	24
Cancerous prostate	134	40	37	1	6
Cerebellum	17	9	1	0	6
Colon	21	11	4	0	0
Dystrophic muscle	41	18	8	0	1
Fetal brain	70	37	16	0	1
Fetal kidney	227	116	46	1	19
Fetal liver	13	7	2	0	0
Heart	30	15	7	0	1
Hypertrophic prostate	86	23	22	2	2
Kidney	10	7	3	0	0
Large Intestine	21	8	4	0	1
Liver	23	9	6	0	0
Lung	24	12	4	0	1
Lung (cells)	57	38	6	0	4
Lymph ganglia	163	60	23	2	12
Lymphocytes	23	6	4	0	2
Muscle	33	16	6	0	4
Normal prostate	181	61	45	7	11
Ovary	90	57	12	1	2
Pancreas	48	11	6	0	1
Placenta	24	5	1	0	0
Prostate	34	16	4	0	2
Spleen	56	28	10	0	1
Substantia nigra	108	47	27	1	6
Surrenals	15	3	3	1	0
Testis	131	68	25	1	8
Thyroid	17	8	2	0	2
Umbilical cord	55	17	12	1	3
Uterus	28	15	3	0	2
Non tissue-specific	568	48	177	2	28
Total	2677	947	601	23	150

TABLE VI

Description of Transcription Factor Binding Sites present on promoters isolated from SignalTag sequences

Promoter sequence P13H2 (546 bp):

Matrix	Position	Orientation	Score	Length	Sequence
CMYB_01	-502	+	0.983	9	TGTCAGTTG
MYOD_Q6	-501	-	0.981	10	CCCAACTGAC
S8_01	-444	-	0.980	11	AATAGAATTAG
S8_01	-425	+	0.968	11	AACTAAATTAG
DELTAEF1_01	-390	-	0.980	11	GCACACCTCAG
GATA_C	-384	-	0.984	11	AGATAAATCCA
CMYB_01	-349	+	0.958	9	CTTCAGTTG
GATA1_02	-343	+	0.959	14	TTGTAGATAGGACA
GATA_C	-339	+	0.953	11	AGATAGGACAT
TAL1ALPHA47_01	-235	+	0.973	18	CATAACAGATGGTAAG
TAL1BETA47_01	-235	+	0.983	18	CATAACAGATGGTAAG
TAL1BETA1TF2_01	-235	+	0.978	18	CATAACAGATGGTAAG
MYOD_Q6	-232	-	0.954	10	ACCATCTGTT
GATA1_04	-217	-	0.953	13	TCAAGATAAAGTA
IK1_01	-128	+	0.963	13	AGTTGGGAATTCC
IK2_01	-128	+	0.985	12	AGTTGGGAATTC
CREL_01	-123	+	0.962	10	TGGGAATTCC
GATA1_02	-96	+	0.950	14	TCAGTGATATGGCA
SRY_02	-41	-	0.951	12	TAAAACAAAACA
E2F_02	-33	+	0.957	8	TTTAGCGC
MZF1_01	-5	-	0.975	8	TGAGGGGA

Promoter sequence P16B4 (861bp) :

Matrix	Position	Orientation	Score	Length	Sequence
NFY_Q6	-748	-	0.958	11	GGACCAATCAT
MZF1_01	-738	+	0.962	8	CCTGGGGA
CMYB_01	-684	+	0.994	9	TGACCGTTG
VMYB_02	-682	-	0.985	9	TCCAACGGT
STAT_01	-673	+	0.968	9	TTCCTGGAA
STAT_01	-673	-	0.951	9	TTCCAGGAA
MZF1_01	-556	-	0.956	8	TTGGGGGA
IK2_01	-451	+	0.965	12	GAATGGGATTC
MZF1_01	-424	+	0.986	8	AGAGGGGA
SRY_02	-398	-	0.955	12	GAAAACAAAACA
MZF1_01	-216	+	0.960	8	GAAGGGGA
MYOD_Q6	-190	+	0.981	10	AGCATCTGCC
DELTAEF1_01	-176	+	0.958	11	TCCCACCTTCC
S8_01	5	-	0.992	11	GAGGCAATTAT
MZF1_01	16	-	0.986	8	AGAGGGGA

Promoter sequence P29B6 (555 bp) :

Matrix	Position	Orientation	Score	Length	Sequence
ARNT_01	-311	+	0.984	18	GGACTCACGTGCTGCT
NMYC_01	-309	+	0.965	12	ACTCACGTGCTG
USF_01	-309	+	0.985	12	ACTCACGTGCTG
USF_01	-309	-	0.985	12	CAGCACGTGAGT
NMYC_01	-309	-	0.956	12	CAGCACGTGAGT
MYCMAX_02	-309	-	0.972	12	CAGCACGTGAGT
USF_C	-307	+	0.997	8	TCACGTGC
USF_C	-307	-	0.991	8	GCACGTGA
MZF1_01	-292	-	0.968	8	CATGGGGA
ELK1_02	-105	+	0.983	14	CTCTCCGGAAGCCT
CETS1P54_01	-102	+	0.974	10	TCCGGAAGCC
AP1_Q4	-42	-	0.963	11	AGTGAAGTGAAC
AP1FJ_Q2	-42	-	0.961	11	AGTGAAGTGAAC
PADS_C	45	+	1.000	9	TGTGGTCTC

TABLE VII

CLAIMS

1. A purified or isolated nucleic acid comprising the sequence of one of SEQ ID NOs: 38-305 or comprising a sequence complementary thereto.
- 5 2. The nucleic acid of Claim 1, wherein said nucleic acid is recombinant.
3. A purified or isolated nucleic acid comprising at least 10 consecutive bases of the sequence of one of SEQ ID NOs: 38-305 or one of the sequences complementary thereto.
4. A purified or isolated nucleic acid comprising at least 15 consecutive bases of one of the sequences of SEQ ID NOs: 38-305 or one of the sequences complementary thereto.
- 10 5. The nucleic acid of Claim 4, wherein said nucleic acid is recombinant.
6. A purified or isolated nucleic acid of at least 15 bases capable of hybridizing under stringent conditions to the sequence of one of SEQ ID NOs: 38-305 or one of the sequences complementary to the sequences of SEQ ID NOs: 38-305.
- 15 7. The nucleic acid of Claim 6, wherein said nucleic acid is recombinant.
8. A purified or isolated nucleic acid encoding a human gene product, said human gene product having a sequence partially encoded by one of the sequences of SEQ ID NO: 38-305.
- 20 9. A purified or isolated nucleic acid having the sequence of one of SEQ ID NOs: 38-305 or having a sequence complementary thereto.
10. A purified or isolated nucleic acid comprising the nucleotides of one of SEQ ID NOs: 38-305 which encode a signal peptide.
11. A purified or isolated polypeptides comprising a signal peptide encoded by one of the sequences of SEQ ID NOs: 38-305.
- 25 12. A vector encoding a fusion protein comprising a polypeptide and a signal peptide, said vector comprising a first nucleic acid encoding a signal peptide encoded by one of the sequences of SEQ ID NOs: 38-305 operably linked to a second nucleic acid encoding a polypeptide.
- 30 13. A method of directing the extracellular secretion of a polypeptide or the insertion of a polypeptide into the membrane comprising the steps of:

obtaining a vector according to Claim 12; and

introducing said vector into a host cell such that said fusion protein is secreted into the extracellular environment of said host cell or inserted into the membrane of said host cell.

14. A method of importing a polypeptide into a cell comprising contacting said
5 cell with a fusion protein comprising a signal peptide encoded by one of the sequences of SEQ ID NOs: 38-305 operably linked to said polypeptide.

15. A method of making a cDNA encoding a human secretory protein that is partially encoded by one of SEQ ID NOs 38-305, comprising the steps of:

obtaining a cDNA comprising one of the sequences of SEQ ID NOs: 38-305;

10 contacting said cDNA with a detectable probe comprising at least 15 consecutive nucleotides of said sequence of SEQ ID NO: 38-305 or a sequence complementary thereto under conditions which permit said probe to hybridize to said cDNA;

identifying a cDNA which hybridizes to said detectable probe; and

isolating said cDNA which hybridizes to said probe.

15 16. An isolated or purified cDNA encoding a human secretory protein, said human secretory protein comprising the protein encoded by one of SEQ ID NOs 38-305 or a fragment thereof of at least 10 amino acids, said cDNA being obtainable by the method of Claim 15.

17. The cDNA of Claim 16 wherein said cDNA comprises the full protein coding
20 sequence partially included in one of the sequences of SEQ ID NOs: 38-305.

18. A method of making a cDNA comprising one of the sequences of SEQ ID NOs: 38-305, comprising the steps of:

contacting a collection of mRNA molecules from human cells with a first primer capable of hybridizing to the polyA tail of said mRNA;

25 hybridizing said first primer to said polyA tail;

reverse transcribing said mRNA to make a first cDNA strand;

making a second cDNA strand complementary to said first cDNA strand using at least one primer comprising at least 15 nucleotides of one of the sequences of SEQ ID NOs 38-305; and

30 isolating the resulting cDNA comprising said first cDNA strand and said second cDNA strand.

19. An isolated or purified cDNA encoding a human secretory protein, said human secretory protein comprising the protein encoded by one of SEQ ID NOs 38-305 or a fragment thereof of at least 10 amino acids, said cDNA being obtainable by the method of Claim 18.

5 20. The cDNA of Claim 19 wherein said cDNA comprises the full protein coding sequence partially included in one of the sequences of SEQ ID NOs: 38-305.

21. The method of Claim 18, wherein the second cDNA strand is made by:
contacting said first cDNA strand with a first pair of primers, said first pair of primers comprising a second primer comprising at least 15 consecutive nucleotides of one of the
10 sequences of SEQ ID NOs 38-305 and a third primer having a sequence therein which is included within the sequence of said first primer;

performing a first polymerase chain reaction with said first pair of nested primers to generate a first PCR product;

contacting said first PCR product with a second pair of primers, said second pair of
15 primers comprising a fourth primer, said fourth primer comprising at least 15 consecutive nucleotides of said sequence of one of SEQ ID NO:s 38-305, and a fifth primer, said fourth and fifth primers being capable of hybridizing to sequences within said first PCR product; and
performing a second polymerase chain reaction, thereby generating a second PCR product.

20 22. An isolated or purified cDNA encoding a human secretory protein, said human secretory protein comprising the protein encoded by one of SEQ ID NOs 38-305, or a fragment thereof of at least 10 amino acids, said cDNA being obtainable by the method of Claim 21.

23. The cDNA of Claim 22 wherein said cDNA comprises the full protein coding
25 sequence partially included in one of the sequences of SEQ ID NOs: 38-305.

24. The method of Claim 18 wherein the second cDNA strand is made by:
contacting said first cDNA strand with a second primer comprising at least 15
consecutive nucleotides of the sequences of SEQ ID NOs: 38-305;

hybridizing said second primer to said first strand cDNA; and
30 extending said hybridized second primer to generate said second cDNA strand.

25. An isolated or purified cDNA encoding a human secretory protein, said human secretory protein comprising the protein partially encoded by one of SEQ ID NOs 38-305 or comprising a fragment thereof of at least 10 amino acids, said cDNA being obtainable by the method of Claim 24.

5 26. The cDNA of Claim 25, wherein said cDNA comprises the full protein coding sequence partially included in one of the sequences of SEQ ID NOs: 38-305.

27. A method of making a protein comprising one of the sequences of SEQ ID NO: 306-573, comprising the steps of:

10 obtaining a cDNA encoding the full protein sequence partially included in one of the sequences of sequence of SEQ ID NO: 38-305;

inserting said cDNA in an expression vector such that said cDNA is operably linked to a promoter;

introducing said expression vector into a host cell whereby said host cell produces the protein encoded by said cDNA; and

15 isolating said protein.

28. An isolated protein obtainable by the method of Claim 27.

29. A method of obtaining a promoter DNA comprising the steps of:

obtaining DNAs located upstream of the nucleic acids of SEQ ID NO: 38-305 or the sequences complementary thereto;

20 screening said upstream DNAs to identify a promoter capable of directing transcription initiation; and

isolating said DNA comprising said identified promoter.

30. The method of Claim 29, wherein said obtaining step comprises chromosome walking from said nucleic acids of SEQ ID NO: 38-305 or sequences complementary thereto.

25 31. The method of Claim 30, wherein said screening step comprises inserting said upstream sequences into a promoter reporter vector.

32. The method of Claim 30, wherein said screening step comprises identifying motifs in said upstream DNAs which are transcription factor binding sites or transcription start sites.

30 33. An isolated promoter obtainable by the method of Claim 32.

34. An isolated or purified protein comprising one of the sequences of SEQ ID NO: 306-573.

35. In an array of discrete ESTs or fragments thereof of at least 15 nucleotides in length, the improvement comprising inclusion in said array of at least one of the sequences of
5 SEQ ID NOs: 38-305, or one of the sequences complementary to the sequences of SEQ ID NOs: 38-305, or a fragment thereof of at least 15 consecutive nucleotides.

36. The array of Claim 35 including therein at least two of the sequences of SEQ ID NOs: 38-305, the sequences complementary to the sequences of SEQ ID NOs: 38-305, or fragments thereof of at least 15 consecutive nucleotides.

10 37. The array of Claim 35 including therein at least five of the sequences of SEQ ID NOs: 38-305, the sequences complementary to the sequences of SEQ ID NOs: 38-305, or fragments thereof of at least 15 consecutive nucleotides.

1/4

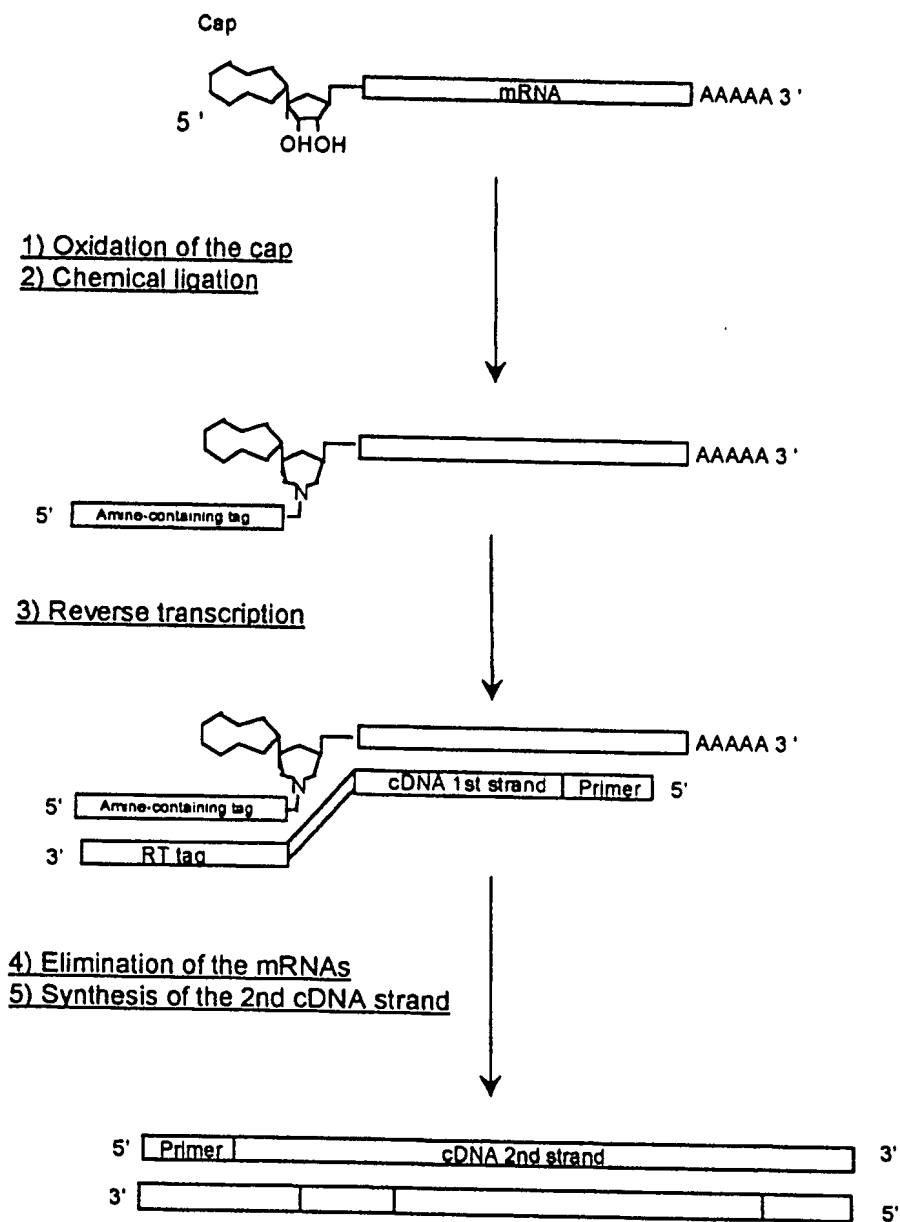


Figure 1

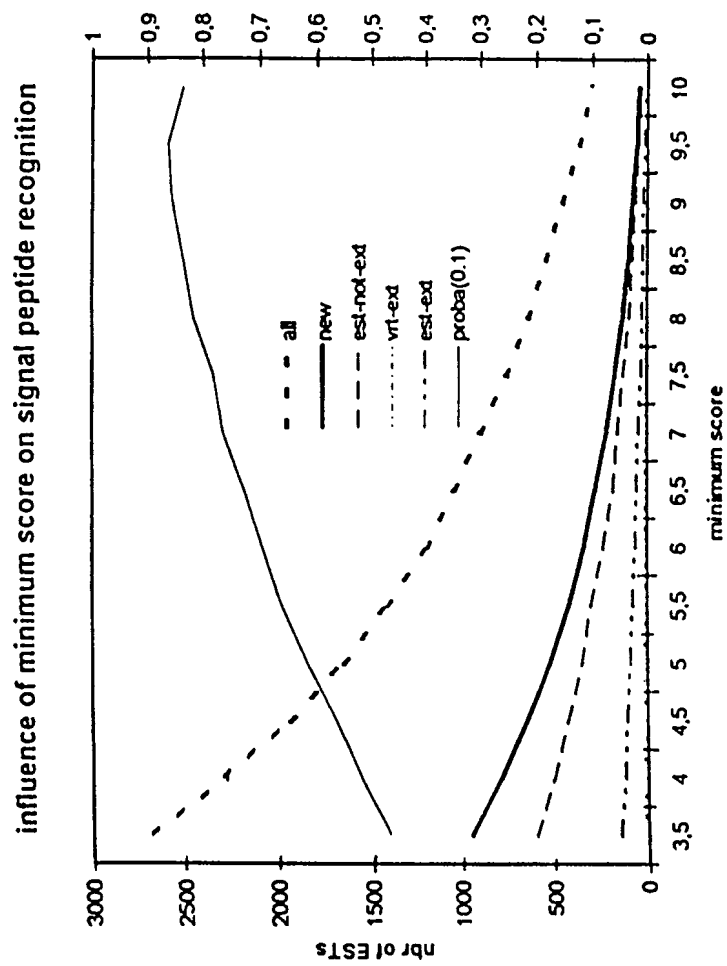


Figure 2

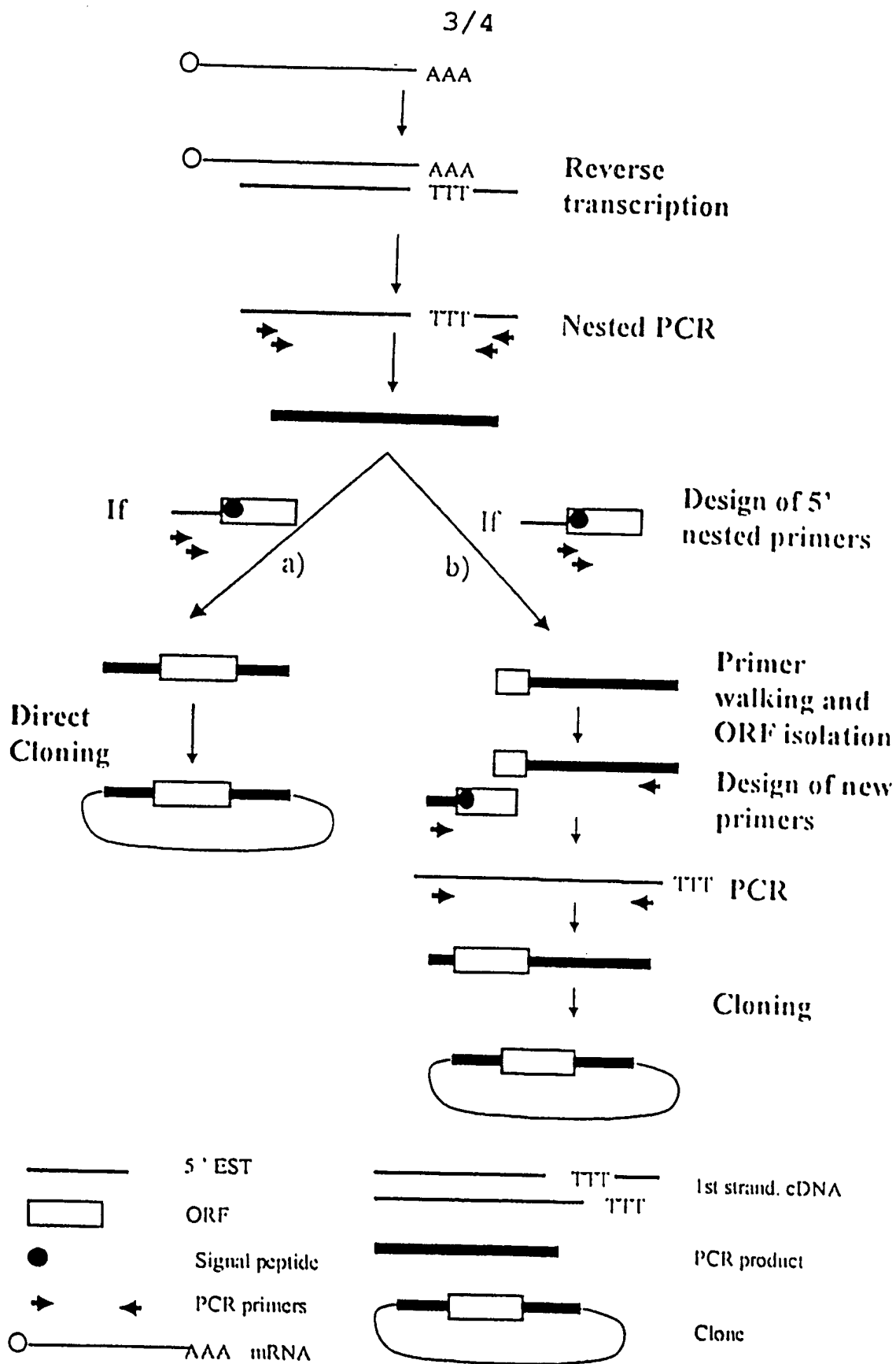
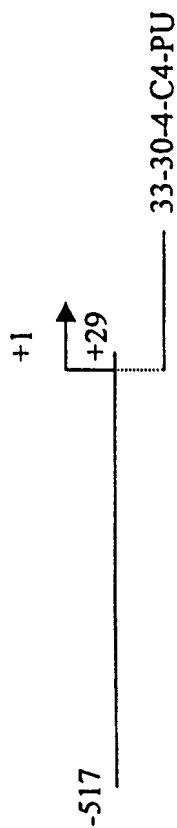
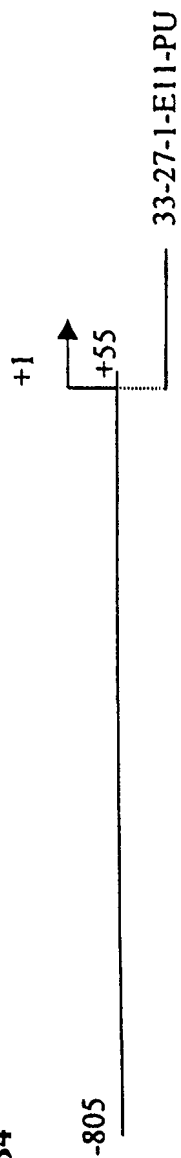


Figure 3

Promoter P13H2



Promoter P15B4



Promoter P29B6

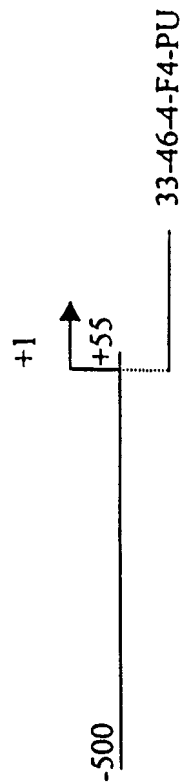


Figure 4

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

- (A) NAME : GENSET SA
- (B) STREET :24, RUE ROYALE
- (C) CITY: PARIS
- (E) COUNTRY : FRANCE
- (F) POSTAL CODE (ZIP) : 75008

(ii) TITLE OF INVENTION: 5' ESTs FOR SECRETED PROTEINS
EXPRESSED IN MUSCLE AND OTHER MESODERMAL TISSUES

(iii) NUMBER OF SEQUENCES: 573

(v) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy Disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: Win95
- (D) SOFTWARE: Word

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 47 base pairs
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: SINGLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Other nucleic acid

(ix) FEATURE:

- (A) NAME/KEY: Cap
- (B) LOCATION: 1
- (D) OTHER INFORMATION: m7Gppp added to 1

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

GGCAUCCUAC UCCCAUCCAA UCCACCCUA ACUCCUCCCA UCUCAC

47

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 46 base pairs
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: SINGLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

GCAUCCUACU CCCAUCCAAU UCCACCCUAA CUCCUCCCAU CUCCAC

46

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25 base pairs
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: SINGLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

ATCAAGAATT CGCACGAGAC CATTAA

25

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25 base pairs
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: SINGLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

TAATGGTCTC GTGCGAATTC TTGAT

25

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25 base pairs
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: SINGLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

CCGACAAGAC CAACGTCAAG GCCGC

25

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25 base pairs
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: SINGLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

TCACCAGCAG GCAGTGGCTT AGGAG

25

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25 base pairs

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

AGTGATTCCT GCTACTTTGG ATGGC

25

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25 base pairs

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

GCTTGGTCTT GTTCTGGAGT TTAGA

25

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25 base pairs

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

TCCAGAATGG GAGACAAGCC AATTT

25

(2) INFORMATION FOR SEQ ID NO: 10:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 25 base pairs
 (B) TYPE: NUCLEIC ACID
 (C) STRANDEDNESS: SINGLE
 (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

AGGGAGGAGG AAACAGCGTG AGTCC

25

(2) INFORMATION FOR SEQ ID NO: 11:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 25 base pairs
 (B) TYPE: NUCLEIC ACID
 (C) STRANDEDNESS: SINGLE
 (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

ATGGGAAAGG AAAAGACTCA TATCA

25

(2) INFORMATION FOR SEQ ID NO: 12:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 25 base pairs
 (B) TYPE: NUCLEIC ACID
 (C) STRANDEDNESS: SINGLE
 (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

AGCAGCAACA ATCAGGACAG CACAG

25

(2) INFORMATION FOR SEQ ID NO: 13:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 25 base pairs
 (B) TYPE: NUCLEIC ACID
 (C) STRANDEDNESS: SINGLE
 (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

ATCAAGAATT CGCACGAGAC CATTA

25

(2) INFORMATION FOR SEQ ID NO: 14:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 67 base pairs
 - (B) TYPE: NUCLEIC ACID
 - (C) STRANDEDNESS: SINGLE
 - (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

ATCGTTGAGA CTCGTACCAG CAGAGTCACG AGAGAGACTA CACGGTACTG GTTTTTTTTTT 60

TTTTTVN 67

(2) INFORMATION FOR SEQ ID NO: 15:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 29 base pairs
 - (B) TYPE: NUCLEIC ACID
 - (C) STRANDEDNESS: SINGLE
 - (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

CCAGCAGAGT CACGAGAGAG ACTACACGG 29

(2) INFORMATION FOR SEQ ID NO: 16:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 25 base pairs
 - (B) TYPE: NUCLEIC ACID
 - (C) STRANDEDNESS: SINGLE
 - (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

CACGAGAGAG ACTACACGGT ACTGG 25

(2) INFORMATION FOR SEQ ID NO: 17:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 526 base pairs
 (B) TYPE: NUCLEIC ACID
 (C) STRANDEDNESS: DOUBLE
 (D) TOPOLOGY: LINEAR
- (ii) MOLECULE TYPE: CDNA
- (vi) ORIGINAL SOURCE:
 (A) ORGANISM: Homo Sapiens
 (F) TISSUE TYPE: Lymph ganglia
- (ix) FEATURE:
 (A) NAME/KEY: other
 (B) LOCATION: complement(261..376)
 (C) IDENTIFICATION METHOD: blastn
 (D) OTHER INFORMATION: identity 96
 region 166..281
 id N70479
 est
- (ix) FEATURE:
 (A) NAME/KEY: other
 (B) LOCATION: complement(380..486)
 (C) IDENTIFICATION METHOD: blastn
 (D) OTHER INFORMATION: identity 97
 region 54..160
 id N70479
 est
- (ix) FEATURE:
 (A) NAME/KEY: other
 (B) LOCATION: complement(110..145)
 (C) IDENTIFICATION METHOD: blastn
 (D) OTHER INFORMATION: identity 94
 region 403..438
 id N70479
 est
- (ix) FEATURE:
 (A) NAME/KEY: other
 (B) LOCATION: complement(196..229)
 (C) IDENTIFICATION METHOD: blastn
 (D) OTHER INFORMATION: identity 94
 region 315..348
 id N70479
 est
- (ix) FEATURE:
 (A) NAME/KEY: sig_peptide
 (B) LOCATION: 90..140
 (C) IDENTIFICATION METHOD: Von Heijne matrix
 (D) OTHER INFORMATION: score 8.2
 seq LLLITAILAVAVG/FP
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

AATATRARAC AGCTACAATA TTCCAGGGCC ARTCACTTGC CATTTCTCAT AACAGCGTCA 60

GAGAGAAAGA ACTGACTGAR ACGTTTGAG ATG AAG AAA GTT CTC CTC CTG ATC 113

Met Lys Lys Val Leu Leu Leu Ile
-15 -10

ACA GCC ATC TTG GCA GTG GCT GTW GGT TTC CCA GTC TCT CAA GAC CAG 161
Thr Ala Ile Leu Ala Val Ala Val Gly Phe Pro Val Ser Gln Asp Gln
-5 1 5

GAA CGA GAA AAA AGA AGT ATC AGT GAC AGC GAT GAA TTA GCT TCA GGR 209
Glu Arg Glu Lys Arg Ser Ile Ser Asp Ser Asp Glu Leu Ala Ser Gly
10 15 20

WTT TTT GTG TTC CCT TAC CCA TAT CCA TTT CGC CCA CTT CCA CCA ATT 257
Xaa Phe Val Phe Pro Tyr Pro Tyr Pro Phe Arg Pro Leu Pro Pro Ile
25 30 35

CCA TTT CCA AGA TTT CCA TGG TTT AGA CGT AAN TTT CCT ATT CCA ATA 305
Pro Phe Pro Arg Phe Pro Trp Phe Arg Arg Xaa Phe Pro Ile Pro Ile
40 45 50 55

CCT GAA TCT GCC CCT ACA ACT CCC CTT CCT AGC GAA AAG TAAACAARAA 354
Pro Glu Ser Ala Pro Thr Thr Pro Leu Pro Ser Glu Lys
60 65

GGAAAAGTCA CRATAAACCT GGTCACCTGA AATTGAAATT GAGCCACTTC CTTGAARAAT 414

CAAAATTCCT GTTAATAAAA RAAAAACAAA TGTAATTGAA ATAGCACACA GCATTCTCTA 474

GTCAATATCT TTAGTGATCT TCTTTAATAA ACATGAAAGC AAAAAAAAAA AA 526

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 17 amino acids
- (B) TYPE: AMINO ACID
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: PROTEIN

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo Sapiens

(ix) FEATURE:

- (A) NAME/KEY: sig_peptide
- (B) LOCATION: 1..17
- (C) IDENTIFICATION METHOD: Von Heijne matrix
- (D) OTHER INFORMATION: score 8.2
seq LLLITAILAVAVG/FP

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

Met Lys Lys Val Leu Leu Leu Ile Thr Ala Ile Leu Ala Val Ala Val
1 5 10 15

Gly

(2) INFORMATION FOR SEQ ID NO: 19:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 822 base pairs
 (B) TYPE: NUCLEIC ACID
 (C) STRANDEDNESS: DOUBLE
 (D) TOPOLOGY: LINEAR
- (ii) MOLECULE TYPE: CDNA
- (vi) ORIGINAL SOURCE:
 (A) ORGANISM: Homo Sapiens
 (D) DEVELOPMENTAL STAGE: Fetal
 (F) TISSUE TYPE: kidney
- (ix) FEATURE:
 (A) NAME/KEY: other
 (B) LOCATION: 260..464
 (C) IDENTIFICATION METHOD: blastn
 (D) OTHER INFORMATION: identity 96
 region 153..357
 id H57434
 est
- (ix) FEATURE:
 (A) NAME/KEY: other
 (B) LOCATION: 118..184
 (C) IDENTIFICATION METHOD: blastn
 (D) OTHER INFORMATION: identity 98
 region 98..164
 id H57434
 est
- (ix) FEATURE:
 (A) NAME/KEY: other
 (B) LOCATION: 56..113
 (C) IDENTIFICATION METHOD: blastn
 (D) OTHER INFORMATION: identity 98
 region 35..92
 id H57434
 est
- (ix) FEATURE:
 (A) NAME/KEY: other
 (B) LOCATION: 454..485
 (C) IDENTIFICATION METHOD: blastn
 (D) OTHER INFORMATION: identity 100
 region 348..379
 id H57434
 est
- (ix) FEATURE:
 (A) NAME/KEY: other
 (B) LOCATION: 118..545
 (C) IDENTIFICATION METHOD: blastn
 (D) OTHER INFORMATION: identity 98
 region 1..428
 id N27248
 est
- (ix) FEATURE:
 (A) NAME/KEY: other

(B) LOCATION: 65..369
(C) IDENTIFICATION METHOD: blastn
(D) OTHER INFORMATION: identity 98
region 41..345
id H94779
est

(ix) FEATURE:

(A) NAME/KEY: other
(B) LOCATION: 61..399
(C) IDENTIFICATION METHOD: blastn
(D) OTHER INFORMATION: identity 99
region 6..344
id H09880
est

(ix) FEATURE:

(A) NAME/KEY: other
(B) LOCATION: 408..458
(C) IDENTIFICATION METHOD: blastn
(D) OTHER INFORMATION: identity 92
region 355..405
id H09880
est

(ix) FEATURE:

(A) NAME/KEY: other
(B) LOCATION: 60..399
(C) IDENTIFICATION METHOD: blastn
(D) OTHER INFORMATION: identity 97
region 56..395
id H29351
est

(ix) FEATURE:

(A) NAME/KEY: other
(B) LOCATION: 393..432
(C) IDENTIFICATION METHOD: blastn
(D) OTHER INFORMATION: identity 90
region 391..430
id H29351
est

(ix) FEATURE:

(A) NAME/KEY: sig_peptide
(B) LOCATION: 346..408
(C) IDENTIFICATION METHOD: Von Heijne matrix
(D) OTHER INFORMATION: score 5.5
seq SFLPSALVIWTS/AF

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

ACTCCTTTTA GCATAGGGGC TTCGGCGCCA GCGGCCAGCG CTAGTCGGTC TGGTAAGTGC	60
CTGATGCCGA GTTCCGTCTC TCGCGTCTTT TCCTGGTCCC AGGCAAAGCG GASGNAGATC	120
CTCAAACGGC CTAGTGCTTC GCGCTTCCGG AGAAAATCAG CGGTCTAATT AATTCCTCTG	180
GTTTGTTGAA GCAGTTACCA AGAATCTTCA ACCCTTTCCC ACAAAGCTA ATTGAGTACA	240

```

CGTTCCTGTT GAGTACACGT TCCTGTTGAT TTACAAAAGG TGCAGGTATG AGCAGGTCTG      300
AAGACTAACA TTTTGTGAAG TTGTAAAACA GAAAACCTGT TAGAA ATG TGG TGG TTT      357
                                     Met Trp Trp Phe
                                     -20

CAG CAA GGC CTC AGT TTC CTT CCT TCA GCC CTT GTA ATT TGG ACA TCT      405
Gln Gln Gly Leu Ser Phe Leu Pro Ser Ala Leu Val Ile Trp Thr Ser
      -15                      -10                      -5

GCT GCT TTC ATA TTT TCA TAC ATT ACT GCA GTA ACA CTC CAC CAT ATA      453
Ala Ala Phe Ile Phe Ser Tyr Ile Thr Ala Val Thr Leu His His Ile
      1                      5                      10                      15

GAC CCG GCT TTA CCT TAT ATC AGT GAC ACT GGT ACA GTA GCT CCA RAA      501
Asp Pro Ala Leu Pro Tyr Ile Ser Asp Thr Gly Thr Val Ala Pro Xaa
      20                      25                      30

AAA TGC TTA TTT GGG GCA ATG CTA AAT ATT GCG GCA GTT TTA TGT CAA      549
Lys Cys Leu Phe Gly Ala Met Leu Asn Ile Ala Ala Val Leu Cys Gln
      35                      40                      45

AAA TAGAAATCAG GAARATAATT CAACTTAAAG AAKTTCATTT CATGACCAA      602
Lys

CTCTTCARAA ACATGTCTTT ACAAGCATAT CTCTTGATT GCTTTCTACA CTGTTGAATT      662
GTCTGGCAAT ATTTCTGCAG TGGAAAATTT GATTIARMTA GTTCTTGACT GATAAATATG      722
GTAAGGTGGG CTTTCCCCC TGTGTAATTG GCTACTATGT CTTACTGAGC CAAGTTGTAW      782
TTTGAAATAA AATGATATGA GAGTGACACA AAAAAAAAAA      822

```

(2) INFORMATION FOR SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 amino acids
- (B) TYPE: AMINO ACID
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: PROTEIN

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo Sapiens

(ix) FEATURE:

- (A) NAME/KEY: sig_peptide
- (B) LOCATION: 1..21
- (C) IDENTIFICATION METHOD: Von Heijne matrix
- (D) OTHER INFORMATION: score 5.5
seq SFLPSALVIWTS/AF

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

```

Met Trp Trp Phe Gln Gln Gly Leu Ser Phe Leu Pro Ser Ala Leu Val
 1           5           10           15

```

Ile Trp Thr Ser Ala
20

(2) INFORMATION FOR SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 405 base pairs
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: DOUBLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo Sapiens
- (F) TISSUE TYPE: Testis

(ix) FEATURE:

- (A) NAME/KEY: other
- (B) LOCATION: complement(103..398)
- (C) IDENTIFICATION METHOD: blastn
- (D) OTHER INFORMATION: identity 96
region 1..296
id AA442893
est

(ix) FEATURE:

- (A) NAME/KEY: sig_peptide
- (B) LOCATION: 185..295
- (C) IDENTIFICATION METHOD: Von Heijne matrix
- (D) OTHER INFORMATION: score 5.9
seq LSYASSALSPCLT/AP

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

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ATCACCTTCT TCTCCATCCT TSTCTGGGCC AGTCCCCARC CCAGTCCCTC TCCTGACCTG      60
CCCAGCCCAA GTCAGCCTTC AGCACGCGCT TTTCTGCACA CAGATATTCC AGGCCTACCT      120
GGCATTCCAG GACCTCCGMA ATGATGCTCC AGTCCCTTAC AAGCGCTTCC TGGATGAGGG      180
TGGC ATG GTG CTG ACC ACC CTC CCC TTG CCC TCT GCC AAC AGC CCT GTG      229
  Met Val Leu Thr Thr Leu Pro Leu Pro Ser Ala Asn Ser Pro Val
    -35                      -30                      -25

AAC ATG CCC ACC ACT GGC CCC AAC AGC CTG AGT TAT GCT AGC TCT GCC      277
Asn Met Pro Thr Thr Gly Pro Asn Ser Leu Ser Tyr Ala Ser Ser Ala
  -20                      -15                      -10

CTG TCC CCC TGT CTG ACC GCT CCA AAK TCC CCC CGG CTT GCT ATG ATG      325
Leu Ser Pro Cys Leu Thr Ala Pro Xaa Ser Pro Arg Leu Ala Met Met
  -5                      1                      5                      10

CCT GAC AAC TAAATATCCT TATCCAAATC AATAAARWRA RAATCCTCCC TCCARAAGGG      384
Pro Asp Asn

TTTCTAAAAA CAAAAA AAA A

```

405

(2) INFORMATION FOR SEQ ID NO: 22:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 37 amino acids
- (B) TYPE: AMINO ACID
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: PROTEIN

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo Sapiens

(ix) FEATURE:

- (A) NAME/KEY: sig_peptide
- (B) LOCATION: 1..37
- (C) IDENTIFICATION METHOD: Von Heijne matrix
- (D) OTHER INFORMATION: score 5.9
seq LSYASSALSPCLT/AP

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

Met Val Leu Thr Thr Leu Pro Leu Pro Ser Ala Asn Ser Pro Val Asn
1 5 10 15
Met Pro Thr Thr Gly Pro Asn Ser Leu Ser Tyr Ala Ser Ser Ala Leu
20 25 30
Ser Pro Cys Leu Thr
35

(2) INFORMATION FOR SEQ ID NO: 23:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 496 base pairs
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: DOUBLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo Sapiens
- (F) TISSUE TYPE: Cancerous prostate

(ix) FEATURE:

- (A) NAME/KEY: other
- (B) LOCATION: 149..331
- (C) IDENTIFICATION METHOD: blastn
- (D) OTHER INFORMATION: identity 98
region 1..183
id AA397994
est

(ix) FEATURE:

- (A) NAME/KEY: other

(B) LOCATION: 328..485
 (C) IDENTIFICATION METHOD: blastn
 (D) OTHER INFORMATION: identity 96
 region 179..336
 id AA397994
 est

(ix) FEATURE:

(A) NAME/KEY: other
 (B) LOCATION: complement(182..496)
 (C) IDENTIFICATION METHOD: blastn
 (D) OTHER INFORMATION: identity 97
 region 14..328
 id AA399680
 est

(ix) FEATURE:

(A) NAME/KEY: sig_peptide
 (B) LOCATION: 196..240
 (C) IDENTIFICATION METHOD: Von Heijne matrix
 (D) OTHER INFORMATION: score 5.5
 seq ILSTVTALTFAXA/LD

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

AAAAAATTGG TCCCAGTTTT CACCCTGCCG CAGGGCTGGC TGGGGAGGGC AGCGGTTTAG	60
ATTAGCCGTG GCCTAGGCCG TTTAACGGGG TGACACGAGC NTGCAGGGCC GAGTCCAAGG	120
CCCGGAGATA GGACCAACCG TCAGGAATGC GAGGAATGTT TTTCTTCGGA CTCTATCGAG	180
GCACACAGAC AGACC ATG GGG ATT CTG TCT ACA GTG ACA GCC TTA ACA TTT	231
Met Gly Ile Leu Ser Thr Val Thr Ala Leu Thr Phe	
-15 -10 -5	
GCC ARA GCC CTG GAC GGC TGC AGA AAT GGC ATT GCC CAC CCT GCA AGT	279
Ala Xaa Ala Leu Asp Gly Cys Arg Asn Gly Ile Ala His Pro Ala Ser	
1 5 10	
GAG AAG CAC AGA CTC GAG AAA TGT AGG GAA CTC GAG ASC ASC CAC TCG	327
Glu Lys His Arg Leu Glu Lys Cys Arg Glu Leu Glu Xaa Xaa His Ser	
15 20 25	
GCC CCA GGA TCA ACC CAS CAC CGA AGA AAA ACA ACC AGA AGA AAT TAT	375
Ala Pro Gly Ser Thr Xaa His Arg Arg Lys Thr Thr Arg Arg Asn Tyr	
30 35 40 45	
TCT TCA GCC TGAAATGAAK CCGGGATCAA ATGGTTGCTG ATCARAGCCC ATATTTAAAT	434
Ser Ser Ala	
TGGAAAAGTC AAATTGASCA TTATTAAATA AAGCTTGTTT AATATGTCTC AAACAAAAAA	494
AA	496

(2) INFORMATION FOR SEQ ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15 amino acids
- (B) TYPE: AMINO ACID
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: PROTEIN

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo Sapiens

(ix) FEATURE:

- (A) NAME/KEY: sig_peptide
- (B) LOCATION: 1..15
- (C) IDENTIFICATION METHOD: Von Heijne matrix
- (D) OTHER INFORMATION: score 5.5
seq ILSTVTALTFAXA/LD

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

```
Met Gly Ile Leu Ser Thr Val Thr Ala Leu Thr Phe Ala Xaa Ala
  1             5             10             15
```

(2) INFORMATION FOR SEQ ID NO: 25:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 623 base pairs
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: DOUBLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo Sapiens
- (F) TISSUE TYPE: Testis

(ix) FEATURE:

- (A) NAME/KEY: sig_peptide
- (B) LOCATION: 49..96
- (C) IDENTIFICATION METHOD: Von Heijne matrix
- (D) OTHER INFORMATION: score 10.1
seq LVLTLCTLPLAVA/SA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

```
AAAGATCCCT GCAGCCCGGC AGGAGAGAAG GCTGAGCCTT CTGGCGTC ATG GAG AGG      57
                                     Met Glu Arg
                                     -15

CTC GTC CTA ACC CTG TGC ACC CTC CCG CTG GCT GTG GCG TCT GCT GGC      105
Leu Val Leu Thr Leu Cys Thr Leu Pro Leu Ala Val Ala Ser Ala Gly
      -10             -5             1

TGC GCC ACG ACG CCA GCT CGC AAC CTG AGC TGC TAC CAG TGC TTC AAG      153
Cys Ala Thr Thr Pro Ala Arg Asn Leu Ser Cys Tyr Gln Cys Phe Lys
  5             10             15

GTC AGC AGC TGG ACG GAG TGC CCG CCC ACC TGG TGC AGC CCG CTG GAC      201
```

Val	Ser	Ser	Trp	Thr	Glu	Cys	Pro	Pro	Thr	Trp	Cys	Ser	Pro	Leu	Asp	
20					25					30					35	
CAA	GTC	TGC	ATC	TCC	AAC	GAG	GTG	GTG	GTC	TCT	TTT	AAA	TGG	AGT	GTA	249
Gln	Val	Cys	Ile	Ser	Asn	Glu	Val	Val	Val	Ser	Phe	Lys	Trp	Ser	Val	
				40					45					50		
CGC	GTC	CTG	CTC	AGC	AAA	CGC	TGT	GCT	CCC	AGA	TGT	CCC	AAC	GAC	AAC	297
Arg	Val	Leu	Leu	Ser	Lys	Arg	Cys	Ala	Pro	Arg	Cys	Pro	Asn	Asp	Asn	
				55				60					65			
ATG	AAK	TTC	GAA	TGG	TCG	CCG	GCC	CCC	ATG	GTG	CAA	GGC	GTG	ATC	ACC	345
Met	Xaa	Phe	Glu	Trp	Ser	Pro	Ala	Pro	Met	Val	Gln	Gly	Val	Ile	Thr	
		70					75					80				
AGG	CGC	TGC	TGT	TCC	TGG	GCT	CTC	TGC	AAC	AGG	GCA	CTG	ACC	CCA	CAG	393
Arg	Arg	Cys	Cys	Ser	Trp	Ala	Leu	Cys	Asn	Arg	Ala	Leu	Thr	Pro	Gln	
	85					90					95					
GAG	GGG	CGC	TGG	GCC	CTG	CRA	GGG	GGG	CTC	CTG	CTC	CAG	GAC	CCT	TCG	441
Glu	Gly	Arg	Trp	Ala	Leu	Xaa	Gly	Gly	Leu	Leu	Leu	Gln	Asp	Pro	Ser	
100				105					110						115	
AGG	GGC	ARA	AAA	ACC	TGG	GTG	CGG	CCA	CAG	CTG	GGG	CTC	CCA	CTC	TGC	489
Arg	Gly	Xaa	Lys	Thr	Trp	Val	Arg	Pro	Gln	Leu	Gly	Leu	Pro	Leu	Cys	
				120				125					130			
CTT	CCC	AWT	TCC	AAC	CCC	CTC	TGC	CCA	RGG	GAA	ACC	CAG	GAA	GGA		534
Leu	Pro	Xaa	Ser	Asn	Pro	Leu	Cys	Pro	Xaa	Glu	Thr	Gln	Glu	Gly		
			135				140					145				
TAACACTGTG	GGTGCCCCCA	CCTGTGCATT	GGGACCACRA	CTTCACCCTC	TTGGARACAA											594
TAAACTCTCA	TGCCCCCAAA	AAAAAAAAA														623

(2) INFORMATION FOR SEQ ID NO: 26:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 16 amino acids
- (B) TYPE: AMINO ACID
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: PROTEIN

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo Sapiens

(ix) FEATURE:

- (A) NAME/KEY: sig_peptide
- (B) LOCATION: 1..16
- (C) IDENTIFICATION METHOD: Von Heijne matrix
- (D) OTHER INFORMATION: score 10.1
seq LVLTLCPLPLAVA/SA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

Met	Glu	Arg	Leu	Val	Leu	Thr	Leu	Cys	Thr	Leu	Pro	Leu	Ala	Val	Ala
1					5				10					15	

(2) INFORMATION FOR SEQ ID NO: 27:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 848 base pairs
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: DOUBLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo Sapiens
- (D) DEVELOPMENTAL STAGE: Fetal
- (F) TISSUE TYPE: kidney

(ix) FEATURE:

- (A) NAME/KEY: sig_peptide
- (B) LOCATION: 32..73
- (C) IDENTIFICATION METHOD: Von Heijne matrix
- (D) OTHER INFORMATION: score 10.7
seq LWLLFFLVTAIHA/EL

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

```

AACTTTGCCT TGTGTTTTCC ACCCTGAAAG A ATG TTG TGG CTG CTC TTT TTT CTG      55
                                Met Leu Trp Leu Leu Phe Phe Leu
                                -10

GTG ACT GCC ATT CAT GCT GAA CTC TGT CAA CCA GGT GCA GAA AAT GCT      103
Val Thr Ala Ile His Ala Glu Leu Cys Gln Pro Gly Ala Glu Asn Ala
-5                               1                               5                               10

TTT AAA GTG AGA CTT AGT ATC AGA ACA GCT CTG GGA GAT AAA GCA TAT      151
Phe Lys Val Arg Leu Ser Ile Arg Thr Ala Leu Gly Asp Lys Ala Tyr
15                               20                               25

GCC TGG GAT ACC AAT GAA GAA TAC CTC TTC AAA GCG ATG GTA GCT TTC      199
Ala Trp Asp Thr Asn Glu Glu Tyr Leu Phe Lys Ala Met Val Ala Phe
30                               35                               40

TCC ATG AGA AAA GTT CCC AAC AGA GAA GCA ACA GAA ATT TCC CAT GTC      247
Ser Met Arg Lys Val Pro Asn Arg Glu Ala Thr Glu Ile Ser His Val
45                               50                               55

CTA CTT TGC AAT GTA ACC CAG AGG GTA TCA TTC TGG TTT GTG GTT ACA      295
Leu Leu Cys Asn Val Thr Gln Arg Val Ser Phe Trp Phe Val Val Thr
60                               65                               70

GAC CCT TCA AAA AAT CAC ACC CTT CCT GCT GTT GAG GTG CAA TCA GCC      343
Asp Pro Ser Lys Asn His Thr Leu Pro Ala Val Glu Val Gln Ser Ala
75                               80                               85                               90

ATA AGA ATG AAC AAG AAC CGG ATC AAC AAT GCC TTC TTT CTA AAT GAC      391
Ile Arg Met Asn Lys Asn Arg Ile Asn Asn Ala Phe Phe Leu Asn Asp
95                               100                               105

CAA ACT CTG GAA TTT TTA AAA ATC CCT TCC ACA CTT GCA CCA CCC ATG      439

```

Gln Thr Leu Glu Phe Leu Lys Ile Pro Ser Thr Leu Ala Pro Pro Met	
110 115 120	
GAC CCA TCT GTG CCC ATC TGG ATT ATT ATA TTT GGT GTG ATA TTT TGC	487
Asp Pro Ser Val Pro Ile Trp Ile Ile Ile Phe Gly Val Ile Phe Cys	
125 130 135	
ATC ATC ATA GTT GCA ATT GCA CTA CTG ATT TTA TCA GGG ATC TGG CAA	535
Ile Ile Ile Val Ala Ile Ala Leu Leu Ile Leu Ser Gly Ile Trp Gln	
140 145 150	
CGT ADA ARA AAG AAC AAA GAA CCA TCT GAA GTG GAT GAC GCT GAA RAT	583
Arg Xaa Xaa Lys Asn Lys Glu Pro Ser Glu Val Asp Asp Ala Glu Xaa	
155 160 165 170	
AAK TGT GAA AAC ATG ATC ACA ATT GAA AAT GGC ATC CCC TCT GAT CCC	631
Xaa Cys Glu Asn Met Ile Thr Ile Glu Asn Gly Ile Pro Ser Asp Pro	
175 180 185	
CTG GAC ATG AAG GGA GGG CAT ATT AAT GAT GCC TTC ATG ACA GAG GAT	679
Leu Asp Met Lys Gly Gly His Ile Asn Asp Ala Phe Met Thr Glu Asp	
190 195 200	
GAG AGG CTC ACC CCT CTC TGAAGGGCTG TTGTTCTGCT TCCTCAARAA	727
Glu Arg Leu Thr Pro Leu	
205	
ATTAAACATT TGTTTCTGTG TGA CTGCTGA GCATCCTGAA ATACCAAGAG CAGATCATAT	787
WTTTGTGTTT ACCATTCTTC TTTTGTAATA AATTTTGAAT GTGCTTGAAA AAAAAAAAAA	847
C	848

(2) INFORMATION FOR SEQ ID NO: 28:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 14 amino acids
- (B) TYPE: AMINO ACID
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: PROTEIN

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo Sapiens

(ix) FEATURE:

- (A) NAME/KEY: sig_peptide
- (B) LOCATION: 1..14
- (C) IDENTIFICATION METHOD: Von Heijne matrix
- (D) OTHER INFORMATION: score 10.7
seq LWLLFFLVTAIHA/EL

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

Met Leu Trp Leu Leu Phe Phe Leu Val Thr Ala Ile His Ala
1 5 10

(2) INFORMATION FOR SEQ ID NO: 29:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25 base pairs
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: SINGLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

GGGAAGATGG AGATAGTATT GCCTG

25

(2) INFORMATION FOR SEQ ID NO: 30:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 26 base pairs
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: SINGLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

CTGCCATGTA CATGATAGAG AGATTC

26

(2) INFORMATION FOR SEQ ID NO: 31:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 546 base pairs
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: DOUBLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Genomic DNA

(ix) FEATURE:

- (A) NAME/KEY: promoter
- (B) LOCATION: 1..517

(ix) FEATURE:

- (A) NAME/KEY: transcription start site
- (B) LOCATION: 518

(ix) FEATURE:

- (A) NAME/KEY: TF binding-site
- (B) LOCATION: 17..25
- (C) IDENTIFICATION METHOD: matinspector prediction
- (D) OTHER INFORMATION: name CMYB_01
score 0.983
sequence TGTCAGTTG

(ix) FEATURE:

- (A) NAME/KEY: TF binding-site
- (B) LOCATION: complement(18..27)
- (C) IDENTIFICATION METHOD: matinspector prediction
- (D) OTHER INFORMATION: name MYOD_Q6
score 0.961
sequence CCCAACTGAC

(ix) FEATURE:

- (A) NAME/KEY: TF binding-site
- (B) LOCATION: complement(75..85)
- (C) IDENTIFICATION METHOD: matinspector prediction
- (D) OTHER INFORMATION: name S8_01
score 0.960
sequence AATAGAATTAG

(ix) FEATURE:

- (A) NAME/KEY: TF binding-site
- (B) LOCATION: 94..104
- (C) IDENTIFICATION METHOD: matinspector prediction
- (D) OTHER INFORMATION: name S8_01
score 0.966
sequence AACTAAATTAG

(ix) FEATURE:

- (A) NAME/KEY: TF binding-site
- (B) LOCATION: complement(129..139)
- (C) IDENTIFICATION METHOD: matinspector prediction
- (D) OTHER INFORMATION: name DELTAEF1_01
score 0.960
sequence GCACACCTCAG

(ix) FEATURE:

- (A) NAME/KEY: TF binding-site
- (B) LOCATION: complement(155..165)
- (C) IDENTIFICATION METHOD: matinspector prediction
- (D) OTHER INFORMATION: name GATA_C
score 0.964
sequence AGATAAATCCA

(ix) FEATURE:

- (A) NAME/KEY: TF binding-site
- (B) LOCATION: 170..178
- (C) IDENTIFICATION METHOD: matinspector prediction
- (D) OTHER INFORMATION: name CMYB_01
score 0.958
sequence CTTAGTTG

(ix) FEATURE:

- (A) NAME/KEY: TF binding-site
- (B) LOCATION: 176..189
- (C) IDENTIFICATION METHOD: matinspector prediction
- (D) OTHER INFORMATION: name GATA1_02
score 0.959
sequence TTGTAGATAGGACA

(ix) FEATURE:

- (A) NAME/KEY: TF binding-site
- (B) LOCATION: 180..190
- (C) IDENTIFICATION METHOD: matinspector prediction
- (D) OTHER INFORMATION: name GATA_C

score 0.953
sequence AGATAGGACAT

(ix) FEATURE:

- (A) NAME/KEY: TF binding-site
- (B) LOCATION: 284..299
- (C) IDENTIFICATION METHOD: matinspector prediction
- (D) OTHER INFORMATION: name TAL1ALPHAE47_01
score 0.973
sequence CATAACAGATGGTAAG

(ix) FEATURE:

- (A) NAME/KEY: TF binding-site
- (B) LOCATION: 284..299
- (C) IDENTIFICATION METHOD: matinspector prediction
- (D) OTHER INFORMATION: name TAL1BETAE47_01
score 0.983
sequence CATAACAGATGGTAAG

(ix) FEATURE:

- (A) NAME/KEY: TF binding-site
- (B) LOCATION: 284..299
- (C) IDENTIFICATION METHOD: matinspector prediction
- (D) OTHER INFORMATION: name TAL1BETAITF2_01
score 0.978
sequence CATAACAGATGGTAAG

(ix) FEATURE:

- (A) NAME/KEY: TF binding-site
- (B) LOCATION: complement(287..296)
- (C) IDENTIFICATION METHOD: matinspector prediction
- (D) OTHER INFORMATION: name MYOD_Q6
score 0.954
sequence ACCATCTGTT

(ix) FEATURE:

- (A) NAME/KEY: TF binding-site
- (B) LOCATION: complement(302..314)
- (C) IDENTIFICATION METHOD: matinspector prediction
- (D) OTHER INFORMATION: name GATA1_04
score 0.953
sequence TCAAGATAAAGTA

(ix) FEATURE:

- (A) NAME/KEY: TF binding-site
- (B) LOCATION: 393..405
- (C) IDENTIFICATION METHOD: matinspector prediction
- (D) OTHER INFORMATION: name IK1_01
score 0.963
sequence AGTTGGGAATTCC

(ix) FEATURE:

- (A) NAME/KEY: TF binding-site
- (B) LOCATION: 393..404
- (C) IDENTIFICATION METHOD: matinspector prediction
- (D) OTHER INFORMATION: name IK2_01
score 0.985
sequence AGTTGGGAATTC

(ix) FEATURE:

- (A) NAME/KEY: TF binding-site

(B) LOCATION: 396..405
(C) IDENTIFICATION METHOD: matinspector prediction
(D) OTHER INFORMATION: name CREL_01
score 0.962
sequence TGGGAATTCC

(ix) FEATURE:

(A) NAME/KEY: TF binding-site
(B) LOCATION: 423..436
(C) IDENTIFICATION METHOD: matinspector prediction
(D) OTHER INFORMATION: name GATA1_02
score 0.950
sequence TCAGTGATATGGCA

(ix) FEATURE:

(A) NAME/KEY: TF binding-site
(B) LOCATION: complement(478..489)
(C) IDENTIFICATION METHOD: matinspector prediction
(D) OTHER INFORMATION: name SRY_02
score 0.951
sequence TAAACAAAACA

(ix) FEATURE:

(A) NAME/KEY: TF binding-site
(B) LOCATION: 486..493
(C) IDENTIFICATION METHOD: matinspector prediction
(D) OTHER INFORMATION: name E2F_02
score 0.957
sequence TTTAGCGC

(ix) FEATURE:

(A) NAME/KEY: TF binding-site
(B) LOCATION: complement(514..521)
(C) IDENTIFICATION METHOD: matinspector prediction
(D) OTHER INFORMATION: name MZF1_01
score 0.975
sequence TGAGGGGA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

```
TGAGTGCAGT GTTACATGTC AGTTGGGTTA AGTTTGTTAA TGTCATTCAA ATCTTCTATG   60
TCTTGATTTG CCTGCTAATT CTATTATTTT TGGAATAAAA TTAGTTTGAT GGTTCTATTA  120
GTTATTGACT GAGGTGTGCT AATCTCCCAT TATGTGGATT TATCTATTTT TTCAGTTGTA  180
GATAGGACAT TGATAGATAC ATAAGTACCA GGACAAAAGC AGGGAGATCT TTTTCCAAA  240
ATCAGGAGAA AAAAATGACA TCTGGAAAAC CTATAGGGAA AGGCATAACA GATGGTAAGG  300
ATACTTTATC TTGAGTAGGA GAGCCTTCCT GTGGCAACGT GGAGAAGGGA AGAGGTCGTA  360
GAATTGAGGA GTCAGCTCAG TTAGAAGCAG GGAGTTGGGA ATTCCGTTCA TGTGATTTAG  420
CATCAGTGAT ATGGCAAATG TGGGACTAAG GGTAGTGATC AGAGGGTTAA AATTGTGTGT  480
TTTGTTTTAG CGCTGCTGGG GCATCGCCTT GGGTCCCCTC AAACAGATTC CCATGAATCT  540
CTTCAT                                     546
```

(2) INFORMATION FOR SEQ ID NO: 32:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 23 base pairs
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: SINGLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 32:

GTACCAGGGA CTGTGACCAT TGC

23

(2) INFORMATION FOR SEQ ID NO: 33:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: SINGLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 33:

CTGTGACCAT TGCTCCCAAG AGAG

24

(2) INFORMATION FOR SEQ ID NO: 34:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 861 base pairs
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: DOUBLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Genomic DNA

(ix) FEATURE:

- (A) NAME/KEY: promoter
- (B) LOCATION: 1..806

(ix) FEATURE:

- (A) NAME/KEY: transcription start site
- (B) LOCATION: 807

(ix) FEATURE:

- (A) NAME/KEY: TF binding-site
- (B) LOCATION: complement(60..70)
- (C) IDENTIFICATION METHOD: matinspector prediction
- (D) OTHER INFORMATION: name NFY_Q6
score 0.956
sequence GGACCAATCAT

(ix) FEATURE:

- (A) NAME/KEY: TF binding-site
- (B) LOCATION: 70..77
- (C) IDENTIFICATION METHOD: matinspector prediction
- (D) OTHER INFORMATION: name MZF1_01
score 0.962
sequence CCTGGGGA

(ix) FEATURE:

- (A) NAME/KEY: TF binding-site
- (B) LOCATION: 124..132
- (C) IDENTIFICATION METHOD: matinspector prediction
- (D) OTHER INFORMATION: name CMYB_01
score 0.994
sequence TGACCGTTG

(ix) FEATURE:

- (A) NAME/KEY: TF binding-site
- (B) LOCATION: complement(126..134)
- (C) IDENTIFICATION METHOD: matinspector prediction
- (D) OTHER INFORMATION: name VMYB_02
score 0.985
sequence TCCAACGGT

(ix) FEATURE:

- (A) NAME/KEY: TF binding-site
- (B) LOCATION: 135..143
- (C) IDENTIFICATION METHOD: matinspector prediction
- (D) OTHER INFORMATION: name STAT_01
score 0.968
sequence TTCCTGGAA

(ix) FEATURE:

- (A) NAME/KEY: TF binding-site
- (B) LOCATION: complement(135..143)
- (C) IDENTIFICATION METHOD: matinspector prediction
- (D) OTHER INFORMATION: name STAT_01
score 0.951
sequence TTCCAGGAA

(ix) FEATURE:

- (A) NAME/KEY: TF binding-site
- (B) LOCATION: complement(252..259)
- (C) IDENTIFICATION METHOD: matinspector prediction
- (D) OTHER INFORMATION: name MZF1_01
score 0.956
sequence TTGGGGGA

(ix) FEATURE:

- (A) NAME/KEY: TF binding-site
- (B) LOCATION: 357..368
- (C) IDENTIFICATION METHOD: matinspector prediction
- (D) OTHER INFORMATION: name IK2_01
score 0.965
sequence GAATGGGATTTC

(ix) FEATURE:

- (A) NAME/KEY: TF binding-site
- (B) LOCATION: 384..391
- (C) IDENTIFICATION METHOD: matinspector prediction

(D) OTHER INFORMATION: name MZF1_01
score 0.986
sequence AGAGGGGA

(ix) FEATURE:

(A) NAME/KEY: TF binding-site
(B) LOCATION: complement(410..421)
(C) IDENTIFICATION METHOD: matinspector prediction
(D) OTHER INFORMATION: name SRY_02
score 0.955
sequence GAAAACAAAACA

(ix) FEATURE:

(A) NAME/KEY: TF binding-site
(B) LOCATION: 592..599
(C) IDENTIFICATION METHOD: matinspector prediction
(D) OTHER INFORMATION: name MZF1_01
score 0.960
sequence GAAGGGGA

(ix) FEATURE:

(A) NAME/KEY: TF binding-site
(B) LOCATION: 618..627
(C) IDENTIFICATION METHOD: matinspector prediction
(D) OTHER INFORMATION: name MYOD_Q6
score 0.981
sequence AGCATCTGCC

(ix) FEATURE:

(A) NAME/KEY: TF binding-site
(B) LOCATION: 632..642
(C) IDENTIFICATION METHOD: matinspector prediction
(D) OTHER INFORMATION: name DELTAEF1_01
score 0.958
sequence TCCCACCTTCC

(ix) FEATURE:

(A) NAME/KEY: TF binding-site
(B) LOCATION: complement(813..823)
(C) IDENTIFICATION METHOD: matinspector prediction
(D) OTHER INFORMATION: name S8_01
score 0.992
sequence GAGGCAATTAT

(ix) FEATURE:

(A) NAME/KEY: TF binding-site
(B) LOCATION: complement(824..831)
(C) IDENTIFICATION METHOD: matinspector prediction
(D) OTHER INFORMATION: name MZF1_01
score 0.986
sequence AGAGGGGA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 34:

TACTATAGGG CACGCGTGGT CGACGGCCGG GCTGTTCTGG AGCAGAGGGC ATGTCAGTAA 60
TGATTGGTCC CTGGGGAAGG TCTGGCTGGC TCCAGCACAG TGAGGCATTT AGGTATCTCT 120
CGGTGACCGT TGGATTCTTG GAAGCAGTAG CTGTTCTGTT TGGATCTGGT AGGGACAGGG 180

CTCAGAGGGC TAGGCACGAG GGAAGGTCAG AGGAGAAGGS AGGSARGGCC CAGTGAGARG 240
GGAGCATGCC TTCCCCAAC CCTGGCTTSC YCTTGGYAM AGGGCGKTTY TGGGMACTTR 300
AAYTCAGGGC CCAASCAGAA SCACAGGCCC AKTCNTGGCT SMAAGCACAA TAGCCTGAAT 360
GGGATTTTCTAG GTTAGNCAGG GTGAGAGGGG AGGCTCTCTG GCTTAGTTTT GTTTTGT TTTT 420
CCAAATCAAG GTAACCTGCT CCCTTCTGCT ACGGGCCTTG GTCTTGGCTT GTCCTCACCC 480
AGTCGGAACCT CCCTACCACT TTCAGGAGAG TGGTTTTAGG CCCGTGGGGC TGTCTGTTC 540
CAAGCAGTGT GAGAACATGG CTGGTAGAGG CTCTAGCTGT GTGCGGGGCC TGAAGGGGAG 600
TGGGTTCTCG CCCAAAGAGC ATCTGCCCAT TTCCACCTT CCCTTCTCCC ACCAGAAGCT 660
TGCCTGAGCT GTTTGGACAA AAATCCAAAC CCCACTTGGC TACTCTGGCC TGGCTTCAGC 720
TTGGAACCCA ATACCTAGGC TTACAGGCCA TCCTGAGCCA GGGGCCTCTG GAAATTCTCT 780
TCCTGATGGT CCTTTAGGTT TGGGCACAAA ATATAATTGC CTCTCCCCTC TCCCATT TTTT 840
TCTCTTGGGA GCAATGGTCA C 861

(2) INFORMATION FOR SEQ ID NO: 35:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 35:

CTGGGATGGA AGGCACGGTA

20

(2) INFORMATION FOR SEQ ID NO: 36:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 36:

GAGACCACAC AGCTAGACAA

20

(2) INFORMATION FOR SEQ ID NO: 37:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 555 base pairs
 - (B) TYPE: NUCLEIC ACID
 - (C) STRANDEDNESS: DOUBLE
 - (D) TOPOLOGY: LINEAR
- (ii) MOLECULE TYPE: Genomic DNA
- (ix) FEATURE:
 - (A) NAME/KEY: promoter
 - (B) LOCATION: 1..500
- (ix) FEATURE:
 - (A) NAME/KEY: transcription start site
 - (B) LOCATION: 501
- (ix) FEATURE:
 - (A) NAME/KEY: TF binding-site
 - (B) LOCATION: 191..206
 - (C) IDENTIFICATION METHOD: matinspector prediction
 - (D) OTHER INFORMATION: name ARNT_01
score 0.964
sequence GGACTCACGTGCTGCT
- (ix) FEATURE:
 - (A) NAME/KEY: TF binding-site
 - (B) LOCATION: 193..204
 - (C) IDENTIFICATION METHOD: matinspector prediction
 - (D) OTHER INFORMATION: name NMYC_01
score 0.965
sequence ACTCACGTGCTG
- (ix) FEATURE:
 - (A) NAME/KEY: TF binding-site
 - (B) LOCATION: 193..204
 - (C) IDENTIFICATION METHOD: matinspector prediction
 - (D) OTHER INFORMATION: name USF_01
score 0.985
sequence ACTCACGTGCTG
- (ix) FEATURE:
 - (A) NAME/KEY: TF binding-site
 - (B) LOCATION: complement(193..204)
 - (C) IDENTIFICATION METHOD: matinspector prediction
 - (D) OTHER INFORMATION: name USF_01
score 0.985
sequence CAGCACGTGAGT
- (ix) FEATURE:
 - (A) NAME/KEY: TF binding-site
 - (B) LOCATION: complement(193..204)
 - (C) IDENTIFICATION METHOD: matinspector prediction
 - (D) OTHER INFORMATION: name NMYC_01
score 0.956
sequence CAGCACGTGAGT
- (ix) FEATURE:
 - (A) NAME/KEY: TF binding-site
 - (B) LOCATION: complement(193..204)
 - (C) IDENTIFICATION METHOD: matinspector prediction

(D) OTHER INFORMATION: name MYCMAX_02
score 0.972
sequence CAGCACGTGAGT

(ix) FEATURE:

(A) NAME/KEY: TF binding-site
(B) LOCATION: 195..202
(C) IDENTIFICATION METHOD: matinspector prediction
(D) OTHER INFORMATION: name USF_C
score 0.997
sequence TCACGTGC

(ix) FEATURE:

(A) NAME/KEY: TF binding-site
(B) LOCATION: complement(195..202)
(C) IDENTIFICATION METHOD: matinspector prediction
(D) OTHER INFORMATION: name USF_C
score 0.991
sequence GCACGTGA

(ix) FEATURE:

(A) NAME/KEY: TF binding-site
(B) LOCATION: complement(210..217)
(C) IDENTIFICATION METHOD: matinspector prediction
(D) OTHER INFORMATION: name MZF1_01
score 0.968
sequence CATGGGGA

(ix) FEATURE:

(A) NAME/KEY: TF binding-site
(B) LOCATION: 397..410
(C) IDENTIFICATION METHOD: matinspector prediction
(D) OTHER INFORMATION: name ELK1_02
score 0.963
sequence CTCTCCGGAAGCCT

(ix) FEATURE:

(A) NAME/KEY: TF binding-site
(B) LOCATION: 400..409
(C) IDENTIFICATION METHOD: matinspector prediction
(D) OTHER INFORMATION: name CETS1P54_01
score 0.974
sequence TCCGGAAGCC

(ix) FEATURE:

(A) NAME/KEY: TF binding-site
(B) LOCATION: complement(460..470)
(C) IDENTIFICATION METHOD: matinspector prediction
(D) OTHER INFORMATION: name AP1_Q4
score 0.963
sequence AGTGACTGAAC

(ix) FEATURE:

(A) NAME/KEY: TF binding-site
(B) LOCATION: complement(460..470)
(C) IDENTIFICATION METHOD: matinspector prediction
(D) OTHER INFORMATION: name AP1FJ_Q2
score 0.961
sequence AGTGACTGAAC

(ix) FEATURE:

- (A) NAME/KEY: TF binding-site
(B) LOCATION: 547..555
(C) IDENTIFICATION METHOD: matinspector prediction
(D) OTHER INFORMATION: name PADS_C
score 1.000
sequence TGTGGTCTC

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 37:

```
CTATAGGGCA CGCKTGGTCG ACGGCCCCGGG CTGGTCTGGT CTGTKGTGGA GTCGGGTTGA    60
AGGACAGCAT TTGTKACATC TGGTCTACTG CACCTTCCCT CTGCCGTGCA CTTGGCCTTT    120
KAWAAGCTCA GCACCGGTGC CCATCACAGG GCCGGCAGCA CACACATCCC ATTACTCAGA    180
AGGAACTGAC GGA CTCACGT GCTGCTCCGT CCCCATGAGC TCAGTGGACC TGTCTATGTA    240
GAGCAGTCAG ACAGTGCCTG GGATAGAGTG AGAGTTCAGC CAGTAAATCC AAGTGATTGT    300
CATTCCTGTC TGCATTAGTA ACTCCCAACC TAGATGTGAA AACTTAGTTC TTTCTCATAG    360
GTTGCTCTGC CCATGGTCCC ACTGCAGACC CAGGCACTCT CCGGAAGCCT GGAAATCACC    420
CGTGTCTTCT GCCTGCTCCC GCTCACATCC CACACTTGTG TTCAGTCACT GAGTTACAGA    480
TTTTGCCTCC TCAATTTCTC TTGTCTTAGT CCCATCCTCT GTTCCCCTGG CCAGTTTGTC    540
TAGCTGTGTG GTCTC                                         555
```

(2) INFORMATION FOR SEQ ID NO: 38:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 140 base pairs
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: DOUBLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo Sapiens
(F) TISSUE TYPE: Heart

(ix) FEATURE:

- (A) NAME/KEY: sig_peptide
(B) LOCATION: 63..122
(C) IDENTIFICATION METHOD: Von Heijne matrix
(D) OTHER INFORMATION: score 15.8
seq LLLLLLLRHGAQG/KP

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 38:

```
AACATTTGCG GGAACRSAGA GCGGANSNG NGACAGCGGA GGAVSTGGAT AACAGGGGAC    60
CG ATG ATG TGG CGA CCA TCA GTT CTG CTG CTT CTG TTG CTA CTG AGG    107
Met Met Trp Arg Pro Ser Val Leu Leu Leu Leu Leu Leu Arg
-20                      -15                      -10
```

CAC GGG GCC CAG GGG AAG CCA TCC CCA GAC GCA
His Gly Ala Gln Gly Lys Pro Ser Pro Asp Ala
-5 1 5

(2) INFORMATION FOR SEQ ID NO: 39:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 404 base pairs
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: DOUBLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo Sapiens
(D) DEVELOPMENTAL STAGE: Fetal
(F) TISSUE TYPE: kidney

(ix) FEATURE:

- (A) NAME/KEY: sig_peptide
(B) LOCATION: 285..359
(C) IDENTIFICATION METHOD: Von Heijne matrix
(D) OTHER INFORMATION: score 14
seq LAMLALLSPLSLA/QY

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 39:

ACTAGTTAAA	AGTAAGTGGG	AAAAGAGTAA	ACGCGCGACT	CCAGCGCGCG	GCTACCTACG	60
CTTGGTGCTT	GCTTTCTCCA	GCCATCGGAG	ACCAGAGCCG	CCCCCTCTGC	TCGAGAAAGG	120
GGCTCAGCGG	CGGCGGAAGC	GGAGGGGGGAC	CACCGTGGAG	AGCGCGGTCC	CAGCCCGGCC	180
ACTGCGGATC	CCTGNAACCA	AAAAGCTCCT	GCTGCTTCTG	TACCCCGCCT	GTCCCTCCCA	240
GCTGCGCAGG	GCCCCTTCGT	GGGATCATCA	GCCCGAAGAC	AGGG	ATG GAG AGG CCT	296
					Met Glu Arg Pro	
					-25	
CTG TGC TCC CAC CTC TGC AGC TGC CTG GCT ATG CTG GCC CTC CTG TCC	344					
Leu Cys Ser His Leu Cys Ser Cys Leu Ala Met Leu Ala Leu Leu Ser						
-20 -15 -10						
CCC CTG AGC CTG GCA CAG TAT GAC AGC TGG CCC CAD KAM CCC GAG TAC	392					
Pro Leu Ser Leu Ala Gln Tyr Asp Ser Trp Pro Xaa Xaa Pro Glu Tyr						
-5 1 5 10						
TTC CAG CAA CCG	404					
Phe Gln Gln Pro						
15						

(2) INFORMATION FOR SEQ ID NO: 40:

(2) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 231 base pairs
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: DOUBLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo Sapiens
- (F) TISSUE TYPE: Dystrophic muscle

(ix) FEATURE:

- (A) NAME/KEY: sig_peptide
- (B) LOCATION: 67..120
- (C) IDENTIFICATION METHOD: Von Heijne matrix
- (D) OTHER INFORMATION: score 12.3
seq HILFLLLPVAAA/QT

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 40:

```

AACAGTTCCT CTGGACTTCT CTGGACCACA GTCCTCTGCC AGACCCCTGC CAGACCCAG      60
TCCACC ATG ATC CAT CTG GGT CAC ATC CTC TTC CTG CTT TTG CTC CCA      108
  Met Ile His Leu Gly His Ile Leu Phe Leu Leu Leu Leu Pro
                    -15                      -10                      -5

GTG GCT GCA GCT CAG ACG ACT CCA GGA GAG AGA TCA TCA CTC CCT GCC      156
Val Ala Ala Ala Gln Thr Thr Pro Gly Glu Arg Ser Ser Leu Pro Ala
                    1                      5                      10

TTT TAC CCT GGC ACT TCA GGC TCT TGT TCC GGA TGT GGG TCC CTC TCT      204
Phe Tyr Pro Gly Thr Ser Gly Ser Cys Ser Gly Cys Gly Ser Leu Ser
                    15                      20                      25

CTG CCG CTC CTG GCA GGC CTC GTG GCT      231
Leu Pro Leu Leu Ala Gly Leu Val Ala
                    30                      35

```

(2) INFORMATION FOR SEQ ID NO: 41:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 161 base pairs
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: DOUBLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo Sapiens
- (D) DEVELOPMENTAL STAGE: Fetal
- (F) TISSUE TYPE: kidney

(ix) FEATURE:

- (A) NAME/KEY: sig_peptide
- (B) LOCATION: 69..134
- (C) IDENTIFICATION METHOD: Von Heijne matrix
- (D) OTHER INFORMATION: score 12.2

seq LALALGLAQPASA/RR

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 41:

```

ATTTCTCCAT CCTCAGTCTT TGCAAGGCGA CAGCTGTGCC AGCCGGGCTC TGGCAGGCTC      60
CTGGCAGC ATG GCA GTG AAG CTT GGG ACC CTC CTG CTG GCC CTT GCC CTG      110
    Met Ala Val Lys Leu Gly Thr Leu Leu Leu Ala Leu Ala Leu
          -20                      -15                      -10

GGC CTG GCC CAG CCA GCC TCT GCC CGC CGG AAG CTG CTG GTG TTT CTG      158
Gly Leu Ala Gln Pro Ala Ser Ala Arg Arg Lys Leu Leu Val Phe Leu
          -5                      1                      5

CTG
Leu
161

```

(2) INFORMATION FOR SEQ ID NO: 42:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 284 base pairs
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: DOUBLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo Sapiens
- (F) TISSUE TYPE: Kidney

(ix) FEATURE:

- (A) NAME/KEY: sig_peptide
- (B) LOCATION: 63..122
- (C) IDENTIFICATION METHOD: Von Heijne matrix
- (D) OTHER INFORMATION: score 11.9
seq LVLEFLLLSPVEA/QQ

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 42:

```

AAAAAACCTG TGGACGCCGA CCCGGGACCG CCGCTGGCTG GCTGCTGGCT CACTCGACCG      60
TC ATG GAG ACC CTG GGG GCC CTT CTG GTG CTG GAG TTT CTG CTC CTC      107
    Met Glu Thr Leu Gly Ala Leu Leu Val Leu Glu Phe Leu Leu Leu
    -20                      -15                      -10

TCC CCG GTG GAG GCC CAG CAG GCC ACG GAG CAT CGC CTG AAG CCG TGG      155
Ser Pro Val Glu Ala Gln Gln Ala Thr Glu His Arg Leu Lys Pro Trp
    -5                      1                      5                      10

CTG GTG GGC CTG GCT GCG GTA GTC GGC TTC CTG TTC ATC GTC TAT TTG      203
Leu Val Gly Leu Ala Ala Val Val Gly Phe Leu Phe Ile Val Tyr Leu
    15                      20                      25

GTC TTG CTG GCC AAC CGC CTC TGG TGT TCC AAG GCC AGG GCT GAG GAC      251
Val Leu Leu Ala Asn Arg Leu Trp Cys Ser Lys Ala Arg Ala Glu Asp
    30                      35                      40

```

GAG GAG GAG ACC ACG TTC AGA ATG GAG TCC GGG
 Glu Glu Glu Thr Thr Phe Arg Met Glu Ser Gly
 45 50

284

(2) INFORMATION FOR SEQ ID NO: 43:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 233 base pairs
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: DOUBLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo Sapiens
- (D) DEVELOPMENTAL STAGE: Fetal
- (F) TISSUE TYPE: kidney

(ix) FEATURE:

- (A) NAME/KEY: sig_peptide
- (B) LOCATION: 63..110
- (C) IDENTIFICATION METHOD: Von Heijne matrix
- (D) OTHER INFORMATION: score 11.3
seq PLLLSSLLGGSQA/MD

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 43:

AACTCACAGC ACGACCAGAG AACAGGCCTG TCTCAGGCAG GCCCTGCGCC TCCTATGCGG 60
 AG ATG CTA CTG CCA CTG CTG CTG TCM TCG CTG CTG GGC GGG TCC CAG 107
 Met Leu Leu Pro Leu Leu Leu Ser Ser Leu Leu Gly Gly Ser Gln
 -15 -10 -5
 GCT ATG GAT GGG AGA TTC TGG ATA CGA GTG CAG GAG TCA GTG ATG GTG 155
 Ala Met Asp Gly Arg Phe Trp Ile Arg Val Gln Glu Ser Val Met Val
 1 5 10 15
 CCG GAG GGC CTG TGC ATC TCT GTN KCC CTG CTC TTT CTC CTA CCC CCG 203
 Pro Glu Gly Leu Cys Ile Ser Val Xaa Leu Leu Phe Leu Leu Pro Pro
 20 25 30
 ACA AGA CTG GAC AGG GTC TAC CCC AGC CGG 233
 Thr Arg Leu Asp Arg Val Tyr Pro Ser Arg
 35 40

(2) INFORMATION FOR SEQ ID NO: 44:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 439 base pairs
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: DOUBLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo Sapiens
 (D) DEVELOPMENTAL STAGE: Fetal
 (F) TISSUE TYPE: kidney

(ix) FEATURE:

- (A) NAME/KEY: sig_peptide
 (B) LOCATION: 32..73
 (C) IDENTIFICATION METHOD: Von Heijne matrix
 (D) OTHER INFORMATION: score 10.7
 seq LWLLFFLVTAIHA/EL

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 44:

```

AACTTTGCCT TGTGTTTTCC ACCCTGAAAG A ATG TTG TGG CTG CTC TTT TTT      52
                               Met Leu Trp Leu Leu Phe Phe
                               -10

CTG GTG ACT GCC ATT CAT GCT GAA CTC TGT CAA CCA GGT GCA GAA AAT      100
Leu Val Thr Ala Ile His Ala Glu Leu Cys Gln Pro Gly Ala Glu Asn
      -5                      1                      5

GCT TTT AAA GTG AGA CTT AGT ATC AGA ACA GCT CTG GGA GAT AAA GCA      148
Ala Phe Lys Val Arg Leu Ser Ile Arg Thr Ala Leu Gly Asp Lys Ala
      10                      15                      20                      25

TAT GCC TGG GAT ACC AAT GAA GAA TAC CTC TTC AAA GCG ATG GTA GCT      196
Tyr Ala Trp Asp Thr Asn Glu Glu Tyr Leu Phe Lys Ala Met Val Ala
                      30                      35                      40

TTC TCC ATG AGA AAA GTT CCC AAC AGA GAA GCA ACA GAA ATT TCC CAT      244
Phe Ser Met Arg Lys Val Pro Asn Arg Glu Ala Thr Glu Ile Ser His
                      45                      50                      55

GTC CTA CTT TGC AAT GTA ACC CAG AGG GTA TCA TTC TGG TTT GTG GTT      292
Val Leu Leu Cys Asn Val Thr Gln Arg Val Ser Phe Trp Phe Val Val
      60                      65                      70

ACA GAC CCT TCA AAA AAT CAC ACC CTT CCT GCT GTT GAG GTG CAA TCA      340
Thr Asp Pro Ser Lys Asn His Thr Leu Pro Ala Val Glu Val Gln Ser
      75                      80                      85

GCC ATA AGA ATG AAC AAG AAC CGG ATC AAC AAT GCC TTC TTT CTA AAT      388
Ala Ile Arg Met Asn Lys Asn Arg Ile Asn Asn Ala Phe Phe Leu Asn
      90                      95                      100                      105

GAC CAA ACT CTG GAA TTT TTA AAA ATC CCT TCC ACA CTT GCA CCA ACC      436
Asp Gln Thr Leu Glu Phe Leu Lys Ile Pro Ser Thr Leu Ala Pro Thr
      110                      115                      120

CGG
Arg
                                         439

```

(2) INFORMATION FOR SEQ ID NO: 45:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 169 base pairs

(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: DOUBLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo Sapiens
(D) DEVELOPMENTAL STAGE: Fetal
(F) TISSUE TYPE: kidney

(ix) FEATURE:

(A) NAME/KEY: sig_peptide
(B) LOCATION: 20..100
(C) IDENTIFICATION METHOD: Von Heijne matrix
(D) OTHER INFORMATION: score 10.7
seq LPLLCLFLQGATA/VL

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 45:

AGAGATCGCA	GCCCAACCC	ATG GCC GGG TCT CCT AGC CGC GCC GCG GGC CGG	52
		Met Ala Gly Ser Pro Ser Arg Ala Ala Gly Arg	
		-25 -20	
CGA CTG CAG CTT CCC CTG CTG TGC CTC TTC CTC CAG GGC GCC ACT GCC			100
Arg Leu Gln Leu Pro Leu Leu Cys Leu Phe Leu Gln Gly Ala Thr Ala			
-15	-10	-5	
GTC CTC TTT GCT GTC TTT GTC CGC TAC AAC CAC AAA ACC GAC GCT GCC			148
Val Leu Phe Ala Val Phe Val Arg Tyr Asn His Lys Thr Asp Ala Ala			
1 5 10 15			
CTC TGG CAM CGG AAG CTT GGG			169
Leu Trp Xaa Arg Lys Leu Gly			
20			

(2) INFORMATION FOR SEQ ID NO: 46:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 204 base pairs
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: DOUBLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo Sapiens
(F) TISSUE TYPE: Dystrophic muscle

(ix) FEATURE:

(A) NAME/KEY: sig_peptide
(B) LOCATION: 40..156
(C) IDENTIFICATION METHOD: Von Heijne matrix
(D) OTHER INFORMATION: score 10.6
seq ALALLLVLP LLWP/CS

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 46:

ACTGCCCTGC CCTGGCCTGA CCCCAGGCCT AQTGAGTCC ATG AAA TGG CCC TGG 54
 Met Lys Trp Pro Trp
 -35

ACC TGC CTT GCC ATC CTC TGT CCT GGC CCT GTA TTG TCC CCA CCA TGC 102
 Thr Cys Leu Ala Ile Leu Cys Pro Gly Pro Val Leu Ser Pro Pro Cys
 -30 -25 -20

TCT GGT CCA RCG CTT GCC CTA GCC CTG TTG CTA GTC CTG CCA CTG CTA 150
 Ser Gly Pro Xaa Leu Ala Leu Ala Leu Leu Val Leu Pro Leu Leu
 -15 -10 -5

TGG CCC TGC TCT GTT TTT GGC CAT GCC CTG TGC TAM CCT AGC CCT GCC 198
 Trp Pro Cys Ser Val Phe Gly His Ala Leu Cys Xaa Pro Ser Pro Ala
 1 5 10

CGA AGG 204
 Arg Arg
 15

(2) INFORMATION FOR SEQ ID NO: 47:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 351 base pairs
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: DOUBLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo Sapiens
- (D) DEVELOPMENTAL STAGE: Fetal
- (F) TISSUE TYPE: kidney

(ix) FEATURE:

- (A) NAME/KEY: sig_peptide
- (B) LOCATION: 28..96
- (C) IDENTIFICATION METHOD: Von Heijne matrix
- (D) OTHER INFORMATION: score 10
seq PLLGLLLSLPAGA/DV

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 47:

AACCGAGCTG GATTTGTATG TTGCACC ATG CCT TCT TGG ATC GGG GCT GTG ATT 54
 Met Pro Ser Trp Ile Gly Ala Val Ile
 -20 -15

CTT CCC CTC TTG GGG CTG CTG CTC TCC CTC CCC GCC GGG GCG GAT GTG 102
 Leu Pro Leu Leu Gly Leu Leu Leu Ser Leu Pro Ala Gly Ala Asp Val
 -10 -5 1

ARG GCT CGG AGC TGC GGA GAG GTC CGC CAG GCG TAC GGT GCC AAG GGA 150
 Lys Ala Arg Ser Cys Gly Glu Val Arg Gln Ala Tyr Gly Ala Lys Gly
 5 10 15

TTC AGC CTG GCG GAC ATC CCC TAC CAG GAG ATC GCA KGG GAA CAC TTA	198
Phe Ser Leu Ala Asp Ile Pro Tyr Gln Glu Ile Ala Xaa Glu His Leu	
20 25 30	
AGA ATC TGT CCT CAG GAA TAT ACA TGC TGC ACC ACA GAA ATG GAR GAC	246
Arg Ile Cys Pro Gln Glu Tyr Thr Cys Cys Thr Thr Glu Met Glu Asp	
35 40 45 50	
AAG TTA AGC CAA CAA AGC AAA CTC GAA TTT GAA AAC CTT GTG GAA GAG	294
Lys Leu Ser Gln Gln Ser Lys Leu Glu Phe Glu Asn Leu Val Glu Glu	
55 60 65	
ACA AGC CAT TTT GTG CGC ACC ACT TTT GTG TCC AGG CAT AAG AAA TTT	342
Thr Ser His Phe Val Arg Thr Thr Phe Val Ser Arg His Lys Lys Phe	
70 75 80	
GAC GGT AGG	351
Asp Gly Arg	
85	

(2) INFORMATION FOR SEQ ID NO: 48:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 242 base pairs
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: DOUBLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo Sapiens
- (D) DEVELOPMENTAL STAGE: Fetal
- (F) TISSUE TYPE: kidney

(ix) FEATURE:

- (A) NAME/KEY: sig_peptide
- (B) LOCATION: 99..182
- (C) IDENTIFICATION METHOD: Von Heijne matrix
- (D) OTHER INFORMATION: score 10
seq LWLSLLVPSC/LCA/SP

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 48:

ACCACTGTGC CCAGCCATTG TCTATACAGT TTGAATAACA CACTGAAAAA ACAGATCAGT	60
GCATATCTTC CACAATTAAC AATGCATTTG TTTAGAGC ATG TTG CTG CAT TGG GTG	116
Met Leu Leu His Trp Val	
-25	
CGC TCT CAG GMT GDC AGC GAC KCN AAG CTT TGG TTG AGT TTG CTA GTG	164
Arg Ser Gln Xaa Xaa Ser Asp Xaa Lys Leu Trp Leu Ser Leu Leu Val	
-20 -15 -10	
CCA AGT TGT TTA TGT GCC TCC CCT TGG CCC CTT CCT TCC CTG CCA CTC	212
Pro Ser Cys Leu Cys Ala Ser Pro Trp Pro Leu Pro Ser Leu Pro Leu	
-5 1 5 10	

CTT CTT CCT CCC AGC TTG CTG AGC TTG CTG
 Leu Leu Pro Pro Ser Leu Leu Ser Leu Leu
 15 20

242

(2) INFORMATION FOR SEQ ID NO: 49:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 289 base pairs
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: DOUBLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo Sapiens
- (D) DEVELOPMENTAL STAGE: Fetal
- (F) TISSUE TYPE: kidney

(ix) FEATURE:

- (A) NAME/KEY: sig_peptide
- (B) LOCATION: 122..223
- (C) IDENTIFICATION METHOD: Von Heijne matrix
- (D) OTHER INFORMATION: score 9.6
seq LLLFSLLVSPPTC/KV

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 49:

```

AAAACTCTT TCTTCGGCTC GCGAGCTGAG AGGAGCAGGT AGAGGGGCAG AGGCGGGACT   60
GTCGTCTGGG GGAGCCGCCC AGGAGGCTCC TCAGGCCGAC CCCAGACCCT GGCTGGCCAG   120
G ATG AAG TAT CTC CGG CAC CGG CGG CCC AAT GCC ACC CTC ATT CTG GCC   169
  Met Lys Tyr Leu Arg His Arg Arg Pro Asn Ala Thr Leu Ile Leu Ala
                    -30                      -25                      -20
ATC GGC GCT TTC ACC CTC CTC CTC TTC AGT CTG CTA GTG TCA CCA CCC   217
Ile Gly Ala Phe Thr Leu Leu Leu Phe Ser Leu Leu Val Ser Pro Pro
                    -15                      -10                      -5
ACC TGC AAG GTC CAG GAG CAG CCA CCG GCG ATC CCC GAG GCC CTG GCC   265
Thr Cys Lys Val Gln Glu Gln Pro Pro Ala Ile Pro Glu Ala Leu Ala
                   1                      5                      10
TGG CHC ACT CCA CCT ACC CGA TGG
Trp Xaa Thr Pro Pro Thr Arg Trp
   15                      20

```

289

(2) INFORMATION FOR SEQ ID NO: 50:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 406 base pairs
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: DOUBLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo Sapiens
- (D) DEVELOPMENTAL STAGE: Fetal
- (F) TISSUE TYPE: kidney

(ix) FEATURE:

- (A) NAME/KEY: sig_peptide
- (B) LOCATION: 26..130
- (C) IDENTIFICATION METHOD: Von Heijne matrix
- (D) OTHER INFORMATION: score 9.5
seq AMWWLLLWGVLA/WP

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 50:

GCAGGTCCCA GATGTCCAGT TCCAG ATG CCT GGA CCC AGA GTG TGG GGG AAA	52
Met Pro Gly Pro Arg Val Trp Gly Lys	
-35 -30	
TAT CTC TGG AGA AGC CCT CAC TCC AAA GGC TGT CCA GGC GCA ATG TGG	100
Tyr Leu Trp Arg Ser Pro His Ser Lys Gly Cys Pro Gly Ala Met Trp	
-25 -20 -15	
TGG CTG CTT CTC TGG GGA GTC CTC CAG GCT TGG CCA AMC CCG GGG CTC	148
Trp Leu Leu Leu Trp Gly Val Leu Gln Ala Trp Pro Xaa Pro Gly Leu	
-10 -5 1 5	
CGT CCT CTT GGC CCA AGA GCT ACC CCA GCA GCT GAC ATC CCC CGG GTA	196
Arg Pro Leu Gly Pro Arg Ala Thr Pro Ala Ala Asp Ile Pro Arg Val	
10 15 20	
CCC AGA GCC GTA TGG CAA AGG CCA AGA GAG CAG CAC GGA CAT CAA GGC	244
Pro Arg Ala Val Trp Gln Arg Pro Arg Glu Gln His Gly His Gln Gly	
25 30 35	
TCC AGA GGG CTT TGC TGT GAG GCT CGT CTT CCA GGA CTT CGA CCT GGA	292
Ser Arg Gly Leu Cys Cys Glu Ala Arg Leu Pro Gly Leu Arg Pro Gly	
40 45 50	
GCC GTC CCA GGA CTG TGC AGG GGA CTC TRW BAC AAT CTC ATT CGT CGG	340
Ala Val Pro Gly Leu Cys Arg Gly Leu Xaa Xaa Asn Leu Ile Arg Arg	
55 60 65 70	
TTC GGA TCC AAG CCA GTT CTG TGG TCA GCA AGG CTC CCC TCT GGG CAG	388
Phe Gly Ser Lys Pro Val Leu Trp Ser Ala Arg Leu Pro Ser Gly Gln	
75 80 85	
GCC CCC TGG TCA GAG GGA	406
Ala Pro Trp Ser Glu Gly	
90	

(2) INFORMATION FOR SEQ ID NO: 51:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 274 base pairs
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: DOUBLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo Sapiens

(F) TISSUE TYPE: Dystrophic muscle

(ix) FEATURE:

(A) NAME/KEY: sig_peptide

(B) LOCATION: 62..172

(C) IDENTIFICATION METHOD: Von Heijne matrix

(D) OTHER INFORMATION: score 9.2
seq LLAVLLASWRLWA/IK

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 51:

```

AACTGGTGCG GCCGAGTGAC AGTTGACCGG TTTTAACCAA GTGACTGGTT CTAGCCACGT      60
T ATG TGC GGC CCA GCC ATG TTC CCT GCC GGT CCT CCG TGG CCC AGA GTC      109
Met Cys Gly Pro Ala Met Phe Pro Ala Gly Pro Pro Trp Pro Arg Val
   -35                               -30                               -25

CGA GTC GTG CAG GTG CTG TGG GCC CTG CTG GCA GTG CTC CTG GCG TCG      157
Arg Val Val Gln Val Leu Trp Ala Leu Leu Ala Val Leu Leu Ala Ser
   -20                               -15                               -10

TGG AGG CTG TGG GCG ATC AAG GAT TTC CAG GAA TGC ACC TGG CAG GTT      205
Trp Arg Leu Trp Ala Ile Lys Asp Phe Gln Glu Cys Thr Trp Gln Val
   -5                               1                               5                               10

GTC CTG AAC GAG TTT AAG AGG GTA GGC GAG AGT GGT GTG AGC GAC AST      253
Val Leu Asn Glu Phe Lys Arg Val Gly Glu Ser Gly Val Ser Asp Xaa
   15                               20                               25

TCT TTG AGC AAG AGC CCG GGG
Ser Leu Ser Lys Ser Pro Gly      274
   30

```

(2) INFORMATION FOR SEQ ID NO: 52:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 259 base pairs

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: DOUBLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo Sapiens

(F) TISSUE TYPE: Muscle

(ix) FEATURE:

(A) NAME/KEY: sig_peptide

(B) LOCATION: 71..235

(C) IDENTIFICATION METHOD: Von Heijne matrix

(D) OTHER INFORMATION: score 9.2

seq SLLLLSTALNILA/CQ

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 52:

```

ACATATCTTT GCAATTGTGA ACATTCAATC ATTTTCAACA CACGTTTCATG GTTAATATTT      60
CTAGGAAACT ATG CAT AGA AGA AAA CTT CCT TTA ACC AAT AAA AGG CAA      109
      Met His Arg Arg Lys Leu Pro Leu Thr Asn Lys Arg Gln
      -55                -50                -45

CTT CAA AAA MCA TTG AGT AAA TTC ATA TTC AGT GAT GAA TTG TTT AGA      157
Leu Gln Lys Xaa Leu Ser Lys Phe Ile Phe Ser Asp Glu Leu Phe Arg
      -40                -35                -30

AAT ATT CTC TTT AGT TTA AGA ACA TTA AGG ATG ATA CTA TCA CTA CTT      205
Asn Ile Leu Phe Ser Leu Arg Thr Leu Arg Met Ile Leu Ser Leu Leu
      -25                -20                -15

CTG TTG AGC ACT GCA TTG AAT ATC TTA GCC TGC CAA ATA AAT GAA GAA      253
Leu Leu Ser Thr Ala Leu Asn Ile Leu Ala Cys Gln Ile Asn Glu Glu
      -10                -5                1                5

CTG GGG      259
Leu Gly

```

(2) INFORMATION FOR SEQ ID NO: 53:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 250 base pairs
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: DOUBLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo Sapiens
- (F) TISSUE TYPE: Heart

(ix) FEATURE:

- (A) NAME/KEY: sig_peptide
- (B) LOCATION: 182..232
- (C) IDENTIFICATION METHOD: Von Heijne matrix
- (D) OTHER INFORMATION: score 8.3
seq VSALLMAWFGVLS/CV

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 53:

```

AAAACGCCGG GAGCTGCGAG TGTCCAGCTG CGGAGACCCG TGATAATTCTG TTAAC TAATT      60
CAACAAACGG GACCCCTTCTG TGTGCCAGAA ACCGCAAGCA GTTGCTAACC CAGTGGGACA      120
GGCGGATTGG AAGAGCGGGA AGGTCCTGGC CCAGAGCAGT GTGACACTTC CCTCTGTGAC      180
C ATG AAA CTC TGG GTG TCT GCA TTG CTG ATG GCC TGG TTT GGT GTC CTG      229
      Met Lys Leu Trp Val Ser Ala Leu Leu Met Ala Trp Phe Gly Val Leu
      -15                -10                -5

```

AGC TGT GTG CAG GCC GAD HYG
 Ser Cys Val Gln Ala Xaa Xaa
 1 5

250

(2) INFORMATION FOR SEQ ID NO: 54:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 198 base pairs
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: DOUBLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo Sapiens
- (D) DEVELOPMENTAL STAGE: Fetal
- (F) TISSUE TYPE: kidney

(ix) FEATURE:

- (A) NAME/KEY: sig_peptide
- (B) LOCATION: 49..105
- (C) IDENTIFICATION METHOD: Von Heijne matrix
- (D) OTHER INFORMATION: score 8.1
seq LCLVCLLVHTAFR/VV

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 54:

AAGAGCCTGT GCTACTGGAA GGTGGCGTGC CCTCCTCTGG CTGGTACC ATG CAG CTC	57
Met Gln Leu	
CCA CTG GCC CTG TGT CTC GTC TGC CTG CTG GTA CAC ACA GCC TTC CGT	105
Pro Leu Ala Leu Cys Leu Val Cys Leu Leu Val His Thr Ala Phe Arg	
-15 -10 -5	
GTA GTG GAG GGC CAG GGG TGG CAG GCG TTC AAG AAT GAT GCC ACG GAA	153
Val Val Glu Gly Gln Gly Trp Gln Ala Phe Lys Asn Asp Ala Thr Glu	
1 5 10 15	
ATC ATC CCC GAG CTC GGA GAG TAC CCC GAG CCT CCA CCG GAA CGG	198
Ile Ile Pro Glu Leu Gly Glu Tyr Pro Glu Pro Pro Pro Glu Arg	
20 25 30	

(2) INFORMATION FOR SEQ ID NO: 55:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 206 base pairs
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: DOUBLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo Sapiens

(F) TISSUE TYPE: Muscle

(ix) FEATURE:

(A) NAME/KEY: sig_peptide
 (B) LOCATION: 99..191
 (C) IDENTIFICATION METHOD: Von Heijne matrix
 (D) OTHER INFORMATION: score 8
 seq ILLCSVAVXLSPS/EP

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 55:

```

CATAGGGTTT CGAAAATTAT CCACACTTTC TATGGTAATA GAATCTGATA TGGTTCACTC      60
TTGGTGTGTG ACATTCTGTG GGTCTGGGTA AATGTATA ATG TTA TGT ATC CAC CAN      116
                               Met Leu Cys Ile His Xaa
                               -30

KAT AGG ATC ATA CAG GAC AGT TTC ATT GCC CTA AAA ATT CTC TTA TGT      164
Xaa Arg Ile Ile Gln Asp Ser Phe Ile Ala Leu Lys Ile Leu Leu Cys
-25                               -20                               -15                               -10

TCT GTC GCT GTA TSM CTG TCT CCC TCC GAG CCC CTG GCG CCG      206
Ser Val Ala Val Xaa Leu Ser Pro Ser Glu Pro Leu Ala Pro
                               -5                               1                               5
  
```

(2) INFORMATION FOR SEQ ID NO: 56:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 220 base pairs
 (B) TYPE: NUCLEIC ACID
 (C) STRANDEDNESS: DOUBLE
 (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo Sapiens
 (D) DEVELOPMENTAL STAGE: Fetal
 (F) TISSUE TYPE: kidney

(ix) FEATURE:

(A) NAME/KEY: sig_peptide
 (B) LOCATION: 8..121
 (C) IDENTIFICATION METHOD: Von Heijne matrix
 (D) OTHER INFORMATION: score 7.9
 seq LPFLSLFWPWAPG/AV

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 56:

```

AAGGAGC ATG GGT GGT TTT TTT CCC CCT ACC GAG GTC CGT GAG GTG TGT      49
  Met Gly Gly Phe Phe Pro Pro Thr Glu Val Arg Glu Val Cys
                               -35                               -30                               -25

GCT AAC CAA GGG GCG GCT CAC AAC CGT GAC AGA CTG CCA TTC CTG AGT      97
Ala Asn Gln Gly Ala Ala His Asn Arg Asp Arg Leu Pro Phe Leu Ser
                               -20                               -15                               -10
  
```

CTC TTC TGG CCA TGG GCC CCC GGA GCC GTG AGC GTC GGG CAG GCG CGG	145
Leu Phe Trp Pro Trp Ala Pro Gly Ala Val Ser Val Gly Gln Ala Arg	
-5 1 5	
TAC AGA ACA CCA ACG ACA KSA GCG CCC TCA GCA AGC GTT CCC TGG CCG	193
Tyr Arg Thr Pro Thr Thr Xaa Ala Pro Ser Ala Ser Val Pro Trp Pro	
10 15 20	
CGC GCG GGT ACG TGC AGG ACC CCT ACG	220
Arg Ala Gly Thr Cys Arg Thr Pro Thr	
25 30	

(2) INFORMATION FOR SEQ ID NO: 57:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 131 base pairs
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: DOUBLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo Sapiens
- (F) TISSUE TYPE: Heart

(ix) FEATURE:

- (A) NAME/KEY: sig_peptide
- (B) LOCATION: 21..110
- (C) IDENTIFICATION METHOD: Von Heijne matrix
- (D) OTHER INFORMATION: score 7.9
seq HLWILLFFSFCWM/SR

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 57:

ACTTCCCTAT TATTCCTGAA ATG AAA TTA TTT TAC AAC CAG CTC GTT TCA GAA	53
Met Lys Leu Phe Tyr Asn Gln Leu Val Ser Glu	
-30 -25 -20	
ACA AAA CAT GAT TTT GCA CAT TTG TGG ATT TTG TTG TTA TTC TCA TTT	101
Thr Lys His Asp Phe Ala His Leu Trp Ile Leu Leu Leu Phe Ser Phe	
-15 -10 -5	
TGT TGG ATG TCT AGA AGC TTT TTT TTT TTT	131
Cys Trp Met Ser Arg Ser Phe Phe Phe Phe	
1 5	

(2) INFORMATION FOR SEQ ID NO: 58:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 179 base pairs
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: DOUBLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo Sapiens
- (F) TISSUE TYPE: Dystrophic muscle

(ix) FEATURE:

- (A) NAME/KEY: sig_peptide
- (B) LOCATION: 111..170
- (C) IDENTIFICATION METHOD: Von Heijne matrix
- (D) OTHER INFORMATION: score 7.9
seq LLFFHILFHSCFS/HL

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 58:

```

ACCTTTAAGA TTACCTGTAT AATAAATGTG TGCAGACACC ATCCAAAAG GTGTAAAAA   60
TTGCAAAGGA AAAATAAATA CTGGCCAACA CAGTGTCTT AAAAGTACCC ATG CCT   116
                                   Met Pro
                                   -20
AGT GAG TCC CCT CCC TTG CTG TTC TTT CAC ATT CTG TTC CAT AGC TGT   164
Ser Glu Ser Pro Pro Leu Leu Phe Phe His Ile Leu Phe His Ser Cys
      -15                      -10                      -5
TTC TCC CAC CTC TTG   179
Phe Ser His Leu Leu
      1

```

(2) INFORMATION FOR SEQ ID NO: 59:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 362 base pairs
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: DOUBLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo Sapiens
- (D) DEVELOPMENTAL STAGE: Fetal
- (F) TISSUE TYPE: kidney

(ix) FEATURE:

- (A) NAME/KEY: sig_peptide
- (B) LOCATION: 18..221
- (C) IDENTIFICATION METHOD: Von Heijne matrix
- (D) OTHER INFORMATION: score 7.9
seq LLCSALAWQQSLS/GK

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 59:

```

ATAAACAGGA AAGCACT ATG TCT TCA ATG TGG TCT GAA TAT ACA ATT GGT   50
               Met Ser Ser Met Trp Ser Glu Tyr Thr Ile Gly
               -65                      -60
GGG GTG AAG ATT TAC TTT CCT TAT AAA GCT TAC CCG TCA CAG CTT GCT   98

```

Gly Val Lys Ile Tyr Phe Pro Tyr Lys Ala Tyr Pro Ser Gln Leu Ala	
-55 -50 -45	
ATG ATG AAT TCT ATT CTC AGA GGA TTA AAC AGC AAG CAA CAT TGT TTG	146
Met Met Asn Ser Ile Leu Arg Gly Leu Asn Ser Lys Gln His Cys Leu	
-40 -35 -30	
TTG GAG AGT CCC ACA GGA AGT GGA AAA AGC TTA GCC TTA CTT TGT TCT	194
Leu Glu Ser Pro Thr Gly Ser Gly Lys Ser Leu Ala Leu Leu Cys Ser	
-25 -20 -15 -10	
GCT TTA GCA TGG CAA CAA TCT CTT AGT GGG AAA CCA GCA GAT GAG GGC	242
Ala Leu Ala Trp Gln Gln Ser Leu Ser Gly Lys Pro Ala Asp Glu Gly	
-5 1 5	
GTA AGT GAA AAA GCT GAA GTA CAA TTG TCA TGT TGT TGT GCA TGC CAT	290
Val Ser Glu Lys Ala Glu Val Gln Leu Ser Cys Cys Cys Ala Cys His	
10 15 20	
TCA AAG GAT TTT ACA AAC AAT GAC ATG AAC CAA GGA ACT TCA CGT CAT	338
Ser Lys Asp Phe Thr Asn Asn Asp Met Asn Gln Gly Thr Ser Arg His	
25 30 35	
TTC AAC TAT CCA AGC ACA CCA CGG	362
Phe Asn Tyr Pro Ser Thr Pro Arg	
40 45	

(2) INFORMATION FOR SEQ ID NO: 60:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 129 base pairs
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: DOUBLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo Sapiens
- (D) DEVELOPMENTAL STAGE: Fetal
- (F) TISSUE TYPE: kidney

(ix) FEATURE:

- (A) NAME/KEY: sig_peptide
- (B) LOCATION: 19..102
- (C) IDENTIFICATION METHOD: Von Heijne matrix
- (D) OTHER INFORMATION: score 7.8
seq FVRFLGFVSCQLQS/DP

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 60:

TAGCTATTTT CAGCGCTT ATG GCT CTG TTC TTG GAG TTA TTT CTA AAT TCT	51
Met Ala Leu Phe Leu Glu Leu Phe Leu Asn Ser	
-25 -20	
TAT TCT CTT TTG TTT GTA AGG TTT CTT GGC TTT GTT TCC TGT TTG CAG	99
Tyr Ser Leu Leu Phe Val Arg Phe Leu Gly Phe Val Ser Cys Leu Gln	
-15 -10 -5	

TCT GAT CCC ATT TGC TCT TTT TTT TTT TTT
 Ser Asp Pro Ile Cys Ser Phe Phe Phe Phe
 1 5

129

(2) INFORMATION FOR SEQ ID NO: 61:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 329 base pairs
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: DOUBLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo Sapiens
- (D) DEVELOPMENTAL STAGE: Fetal
- (F) TISSUE TYPE: kidney

(ix) FEATURE:

- (A) NAME/KEY: sig_peptide
- (B) LOCATION: 114..185
- (C) IDENTIFICATION METHOD: Von Heijne matrix
- (D) OTHER INFORMATION: score 7.8
seq LMAGSSLSAGVSG/ED

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 61:

ATACTTCAAA TCTTGAATTA AATGAAGAAA TTTATTTTAC TGATTCTCTT GAAATAAAGA 60
 GAAATGAAAA TTTTCCAAAG GATTATGTGA AATTTTCAGA TGAAGAAGAA TTT ATG 116
 Met
 AAT GAA GAT GAG AAG GAA ATG AAG GAA ATT CTA ATG GCA GGA AGT AGT 164
 Asn Glu Asp Glu Lys Glu Met Lys Glu Ile Leu Met Ala Gly Ser Ser
 -20 -15 -10
 TTA TCA GCT GGA GTT AGT GGG GAA GAT AAA ACC GAG ATA TTG AAT CCC 212
 Leu Ser Ala Gly Val Ser Gly Glu Asp Lys Thr Glu Ile Leu Asn Pro
 -5 1 5
 ACT CCA SCG ATG GCC AAA TCT CTG ACC ATA GAC TGT CTG GAA TTG GCA 260
 Thr Pro Xaa Met Ala Lys Ser Leu Thr Ile Asp Cys Leu Glu Leu Ala
 10 15 20 25
 TTA CCC CCT GAA CTG GCT TTT CAA CTT AAT GAA TTA TTT GGT CCT GTT 308
 Leu Pro Pro Glu Leu Ala Phe Gln Leu Asn Glu Leu Phe Gly Pro Val
 30 35 40
 GGT ATT GAT TCA GGG TCT CTA 329
 Gly Ile Asp Ser Gly Ser Leu
 45

(2) INFORMATION FOR SEQ ID NO: 62:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 247 base pairs
 (B) TYPE: NUCLEIC ACID
 (C) STRANDEDNESS: DOUBLE
 (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

- (vi) ORIGINAL SOURCE:
 (A) ORGANISM: Homo Sapiens
 (F) TISSUE TYPE: Heart

- (ix) FEATURE:
 (A) NAME/KEY: sig_peptide
 (B) LOCATION: 167..229
 (C) IDENTIFICATION METHOD: Von Heijne matrix
 (D) OTHER INFORMATION: score 7.8
 seq IIPLIXXLSLCLC/LW

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 62:

```

CTATACGTGA TAAGTGAATA AAATGTGTCA GAGTGTACTA CTTAGAATTT TCATAGATTG   60
TAAAGATTTT CTATATATTT ATTTGAATTG GTAATTGGTT ATGAGCAGTT TGGTGTAGCT   120
GTTTTTAATT GTACAACAAT TAAGATATCA CCTATATTCT CGAAGA ATG GGA TCA       175
                                   Met Gly Ser
                                   -20
TTC CTT CTA GGA GGG ATT ATC CCT TTA ATA NNT TTN CTT TCT CTT TGT       223
Phe Leu Leu Gly Gly Ile Ile Pro Leu Ile Xaa Xaa Leu Ser Leu Cys
      -15                               -10                               -5
CTT TGT TTA TGG TGG AGA ATA ATT
Leu Cys Leu Trp Trp Arg Ile Ile                                     247
      1                               5

```

(2) INFORMATION FOR SEQ ID NO: 63:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 399 base pairs
 (B) TYPE: NUCLEIC ACID
 (C) STRANDEDNESS: DOUBLE
 (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

- (vi) ORIGINAL SOURCE:
 (A) ORGANISM: Homo Sapiens
 (D) DEVELOPMENTAL STAGE: Fetal
 (F) TISSUE TYPE: kidney

- (ix) FEATURE:
 (A) NAME/KEY: sig_peptide
 (B) LOCATION: 277..369
 (C) IDENTIFICATION METHOD: Von Heijne matrix
 (D) OTHER INFORMATION: score 7.8
 seq VCLLCSGCSCAWS/VG

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 63:

```

ACGAGTGTTA CAGAGGAGAT CTGGTTTCTG GAGGTCTCCA GGATGGGGCT GTAGCCTAAA      60
AGGAAGACTA TGTGAGGCAG CAGGCAAGCA GCAGCAAGTG GAAAGGCTTG GAGATGTGGA      120
GGACGTTATA TGGTACTCAG AGAGCAGCAG TACATGGATG GCAAGTGTGG CGTTGTGCTG      180
CCACCCACTT CCCCATGCCA AAAGCATATA ACTGCTAATC AGTTACCGCA TTTTTTGCTG      240
CCGAATTCGT AAGCAGCCCC AAGAGTTCTC AACAGG ATG CTT CAG GTG GCC ACT      294
                               Met Leu Gln Val Ala Thr
                               -30

ACT AAT TAT TTG GAG TTG GCA CGT GAG GTT AAA CCT GTT TGT CTT CTT      342
Thr Asn Tyr Leu Glu Leu Ala Arg Glu Val Lys Pro Val Cys Leu Leu
-25                               -20                               -15                               -10

TGT AGT GGG TGT TCC TGT GCC TGG AGC GTA GGA TGT GTG TKG GAG TCG      390
Cys Ser Gly Cys Ser Cys Ala Trp Ser Val Gly Cys Val Xaa Glu Ser
                               -5                               1                               5

GAG TCA GAA                                                                399
Glu Ser Glu
10

```

(2) INFORMATION FOR SEQ ID NO: 64:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 240 base pairs
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: DOUBLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo Sapiens
- (D) DEVELOPMENTAL STAGE: Fetal
- (F) TISSUE TYPE: kidney

(ix) FEATURE:

- (A) NAME/KEY: sig_peptide
- (B) LOCATION: 175..228
- (C) IDENTIFICATION METHOD: Von Heijne matrix
- (D) OTHER INFORMATION: score 7.7
seq PFFLALCFPKSTS/QP

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 64:

```

ATTACTTTGT CTAGATCAGG AGATGCTAGT ATATTCTTAG CACTAAGACC CCTCTGAAAT      60
CTGTGCCAAC ATTTAGCCAC CCAGRAGTTG TKCTTTACTA CACCTTTGAG GGTTATGCCC      120
TGTACATGTG CAGCTTAGGG GTTCAAGGAC AATCTCTTTA CACATTTTTG GGTT ATG      177
                               Met

```

TCA CAG CCC CAA AGG
Ser Gln Pro Gln Arg 240
1

(2) INFORMATION FOR SEQ ID NO: 65:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 451 base pairs
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: DOUBLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo Sapiens
(D) DEVELOPMENTAL STAGE: Fetal
(F) TISSUE TYPE: kidney

(ix) FEATURE:

- (A) NAME/KEY: other
(B) LOCATION: 240..335
(C) IDENTIFICATION METHOD: blastn
(D) OTHER INFORMATION: identity 95
region 1..96
id AA270737
est

(ix) FEATURE:

- (A) NAME/KEY: sig_peptide
(B) LOCATION: 236..331
(C) IDENTIFICATION METHOD: Von Heijne matrix
(D) OTHER INFORMATION: score 7.5
seq QCLLCISPPVFC/EG

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 65:

TCTCTTTGC	TGTTTTCATC	AAGATAGTAG	AGCACATCTT	CTTCTCACAG	ACTACAACATA		60
TGTGGTTCAG	CACGAGGCAG	TAGAGGAAAG	TGCCTCGACT	GTGGGAGGCT	TGGSCAARAT		120
CCAAAGACTT	TCTCTCCTTG	TTGCTGGAGT	CGCTAAAAGA	ACAGTTTAAT	AATGCCACAC		180
CCATCCCCAC	CCACAGTTGT	CCCCTATCTC	CAGACCTCAT	TCGCAATGAA	GTAGA ATG		238
					Met		
TCT GAA AGC AGA TTT CAA CCA CAG AAT CAA GGA GGT TCT CTT CAA CTC							286
Ser Glu Ser Arg Phe Gln Pro Gln Asn Gln Gly Gly Ser Leu Gln Leu	-30	-25	-20				
CCT CTT CAG TGC CTA CTA TGT TGC ATT TCT CCC CCT GTG TTT TGT GAA							334
Pro Leu Gln Cys Leu Leu Cys Cys Ile Ser Pro Pro Val Phe Cys Glu	-15	-10	-5				1

GGT AAC TGG TTA TCT TAC TTT TAT GTG CTT CCT GGA TTT GTG TGT GAA	382
Gly Asn Trp Leu Ser Tyr Phe Tyr Val Leu Pro Gly Phe Val Cys Glu	
5 10 15	
TTA CAT AAA CTG GGT ATT TCT TGT TTA ATC CCC CTT TTC TCT GTC TCC	430
Leu His Lys Leu Gly Ile Ser Cys Leu Ile Pro Leu Phe Ser Val Ser	
20 25 30	
CCT TTG GCA GCC TGG ATG GTG	451
Pro Leu Ala Ala Trp Met Val	
35 40	

(2) INFORMATION FOR SEQ ID NO: 66:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 263 base pairs
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: DOUBLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo Sapiens
- (D) DEVELOPMENTAL STAGE: Fetal
- (F) TISSUE TYPE: kidney

(ix) FEATURE:

- (A) NAME/KEY: sig_peptide
- (B) LOCATION: 114..182
- (C) IDENTIFICATION METHOD: Von Heijne matrix
- (D) OTHER INFORMATION: score 7.3
seq SSCLLGLLHLSSQ/FS

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 66:

ATGGAGCAGA GGTCCAGCTG TGGTGAGGAT TGGCACAGTC GTGCTTGTGG GACTCCTCCT	60
TGGTCCAACCT CTAATGCTCA ACCTACACCA TCACCCCTGT GCTTGCTCCT CTA ATG	116
Met	
CCT AAG CAC TGT CAT TCC TTT ATC ACT AGT AGT TGC CTG TTG GGT TTG	164
Pro Lys His Cys His Ser Phe Ile Thr Ser Ser Cys Leu Leu Gly Leu	
-20 -15 -10	
CTC CAT TTG TCC TCA CAG TTT AGC TGC CCT GGA AGG AAA CTC CAC CCT	212
Leu His Leu Ser Ser Gln Phe Ser Cys Pro Gly Arg Lys Leu His Pro	
-5 1 5 10	
GCT CAG AGA CAC ACT GAG GCT GAG ACC CAA GGG AGG CCC CTC TCT GAC	260
Ala Gln Arg His Thr Glu Ala Glu Thr Gln Gly Arg Pro Leu Ser Asp	
15 20 25	
AGG	263
Arg	

(2) INFORMATION FOR SEQ ID NO: 67:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 351 base pairs
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: DOUBLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo Sapiens
- (F) TISSUE TYPE: Dystrophic muscle

(ix) FEATURE:

- (A) NAME/KEY: sig_peptide
- (B) LOCATION: 166..222
- (C) IDENTIFICATION METHOD: Von Heijne matrix
- (D) OTHER INFORMATION: score 7.2
seq FIXFPFLFPFSFS/QT

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 67:

```

ATCTCTCCTT TTTTCCTGTA ACTGTGCTGG TTTTGTTTGT GTCTTCCTCT CATACCCGTT    60
TCTGCATTTT ATCTTTTCTT TCTATTGIGA CTTCAATTCA TTTTTTTTTT AACCTTATCT    120
TTTGTTTCTC TTGTTTATCC CATCCTTTTT GATAAAATCC ATCGC ATG TGT CTT CTT    177
                                   Met Cys Leu Leu

TTT TYC TTT ATT TYC TTT CCT TTC CTT TTY CCT TTT TCT TTC TCC CAA    225
Phe Xaa Phe Ile Xaa Phe Pro Phe Leu Phe Pro Phe Ser Phe Ser Gln
-15                               -5                               1

ACT TTT TCC TTT TCA CAG CAT TGG AAC ACG GGA GGT AGT CAC CCA GAA    273
Thr Phe Ser Phe Ser Gln His Trp Asn Thr Gly Gly Ser His Pro Glu
      5                               10                               15

GAA CTT GAG CGG CCT GGT GCC CAT CCG AGA CTT AAG GCT AGA CCC CAG    321
Glu Leu Glu Arg Pro Gly Ala His Pro Arg Leu Lys Ala Arg Pro Gln
      20                               25                               30

CCT CCT CTG TTC CAT CCC TTT ATT AGC TCT    351
Pro Pro Leu Phe His Pro Phe Ile Ser Ser
      35                               40

```

(2) INFORMATION FOR SEQ ID NO: 68:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 227 base pairs
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: DOUBLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo Sapiens
(F) TISSUE TYPE: Dystrophic muscle

(ix) FEATURE:

(A) NAME/KEY: sig_peptide
(B) LOCATION: 30..104
(C) IDENTIFICATION METHOD: Von Heijne matrix
(D) OTHER INFORMATION: score 7.1
seq LLVASGXAEVSA/QS

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 68:

```

ACGCGCAGAC CCAGCGCCGA GCCCGAGCC ATG GCG TCC GAG CGG MTC CCT AAY      53
                        Met Ala Ser Glu Arg Xaa Pro Asn
                        -25                      -20

AGG CCC GHC TGT CTG CTC GTR GCC AGC GGC GMC GCC GAR GGT GTG TCG      101
Arg Pro Xaa Cys Leu Leu Val Ala Ser Gly Xaa Ala Glu Gly Val Ser
      -15                      -10                      -5

GCC CAG TCC TTC CTC CAS TGT TTC ACG ATG GCC AGC ACC GSC TTC AAC      149
Ala Gln Ser Phe Leu Xaa Cys Phe Thr Met Ala Ser Thr Xaa Phe Asn
      1                      5                      10                      15

CTG CAG GTG GCC AYC CCT GGK GGG AAA GCC ATG GAA TTT GTS GAT GTG      197
Leu Gln Val Ala Xaa Pro Gly Gly Lys Ala Met Glu Phe Val Asp Val
      20                      25                      30

ACT GAS AGC AAT GCA CGC TGG GTG CAA GAC      227
Thr Xaa Ser Asn Ala Arg Trp Val Gln Asp
      35                      40

```

(2) INFORMATION FOR SEQ ID NO: 69:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 327 base pairs
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: DOUBLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo Sapiens
(D) DEVELOPMENTAL STAGE: Fetal
(F) TISSUE TYPE: kidney

(ix) FEATURE:

(A) NAME/KEY: sig_peptide
(B) LOCATION: 160..234
(C) IDENTIFICATION METHOD: Von Heijne matrix
(D) OTHER INFORMATION: score 7.1
seq LAFQLVFLRATSG/SC

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 69:

```

AATTTCAAGT TGTCATAAAA GTTCAGACAA CCATCACTGG ACCTACAGAT TGAGTGATTA      60

```

TTATAGTGGG GATGTCCTTG GGTAGTAAG CCTAAAGGAA GTAATTTCTG TTAAAGGAGA 120

TGTTAGTGGC CATTGTCATC TTAATGTCAA TCTTATCAG ATG TTC CCA GAC TAC 174
Met Phe Pro Asp Tyr
-25

AAA CTG GGT GGG TCA TAT CTC TTA GCA TTT CAA CTG GTA TTT CTC AGA 222
Lys Leu Gly Gly Ser Tyr Leu Leu Ala Phe Gln Leu Val Phe Leu Arg
-20 -15 -10 -5

GCA ACT AGT GGC TCA TGT TCC AAA TAT AGA AGG CAT TTG CAT AAC ATC 270
Ala Thr Ser Gly Ser Cys Ser Lys Tyr Arg Arg His Leu His Asn Ile
1 5 10

AAT GTT AGA CCT GGG CTT GTT AGA CTC TTG GGC TCA TGT ATA CAA AAG 318
Asn Val Arg Pro Gly Leu Val Arg Leu Leu Gly Ser Cys Ile Gln Lys
15 20 25

CAA CCT GGG 327
Gln Pro Gly
30

(2) INFORMATION FOR SEQ ID NO: 70:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 370 base pairs
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: DOUBLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo Sapiens
- (D) DEVELOPMENTAL STAGE: Fetal
- (F) TISSUE TYPE: kidney

(ix) FEATURE:

- (A) NAME/KEY: sig_peptide
- (B) LOCATION: 44..118
- (C) IDENTIFICATION METHOD: Von Heijne matrix
- (D) OTHER INFORMATION: score 7.1
seq LLLXLXLLLIALE/IM

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 70:

AAATGTGTAC ACGCCCAGCT TCCTGCCTGT TACTCTCCAC AGT ATG CGA AGA ATA 55
Met Arg Arg Ile
-25

TCC CTG ACT TCT AGC CCT GTG CGC CTT CTT TTG TDT CTG CWG TTR CTA 103
Ser Leu Thr Ser Ser Pro Val Arg Leu Leu Leu Xaa Leu Xaa Leu Leu
-20 -15 -10

CTA ATA GCC TTG GAG ATC ATG GTT GGT GGT CAC TCT CTT TGC TTC AAC 151
Leu Ile Ala Leu Glu Ile Met Val Gly Gly His Ser Leu Cys Phe Asn
-5 1 5 10

TTC ACT ATA AAA TCA TTG TCC AGA CCT GGA CAG CCC TGG TGT GAA GCG	199
Phe Thr Ile Lys Ser Leu Ser Arg Pro Gly Gln Pro Trp Cys Glu Ala	
15 20 25	
CAT GTC TTC TTG AAT AAA AAT CTT TTC CTT CAG TAC AAC AGT GAC AAC	247
His Val Phe Leu Asn Lys Asn Leu Phe Leu Gln Tyr Asn Ser Asp Asn	
30 35 40	
AAC ATG GTC AAA CCT CTG GGC CTC CTG GGG AAG AAG GTA TAT GCC ACC	295
Asn Met Val Lys Pro Leu Gly Leu Leu Gly Lys Lys Val Tyr Ala Thr	
45 50 55	
AGC ACT TGG GGA GAA TTG ACC CAA ACG CTG GGA GAA GTG GGG CGA GAC	343
Ser Thr Trp Gly Glu Leu Thr Gln Thr Leu Gly Glu Val Gly Arg Asp	
60 65 70 75	
CTC AGG ATG CTC CTT TGT GAC ATC AAA	370
Leu Arg Met Leu Leu Cys Asp Ile Lys	
80	

(2) INFORMATION FOR SEQ ID NO: 71:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 246 base pairs
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: DOUBLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo Sapiens
- (D) DEVELOPMENTAL STAGE: Fetal
- (F) TISSUE TYPE: kidney

(ix) FEATURE:

- (A) NAME/KEY: sig_peptide
- (B) LOCATION: 193..234
- (C) IDENTIFICATION METHOD: Von Heijne matrix
- (D) OTHER INFORMATION: score 7
seq TFLLLLFxNAGRS/LR

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 71:

AAAATATTTT ATATTAGGGA GAGCTCTGTG CTGCCCTTTC CCAAAGCTTT GGTTATTTGA	60
TGGGAGGGGA AGTCTTCTCG AACCTATGTC MGAATATKCC GCTTTGRAAG AGGAGGGTTT	120
TTCTTGAGGC TAGTTTTGTA CCTGCTGTWT CTTTtagAAA TGATTGCTTT ATGGATTAA	180
AAGGTGACCC AA ATG ACT TTT TTA TTA TTA TTA TTT KTT AAT GCT GGG AGG	231
Met Thr Phe Leu Leu Leu Leu Phe Xaa Asn Ala Gly Arg	
-10 -5	
AGT TTG CGT ATG TGT	246
Ser Leu Arg Met Cys	
1	

(2) INFORMATION FOR SEQ ID NO: 72:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 328 base pairs
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: DOUBLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo Sapiens
- (F) TISSUE TYPE: Dystrophic muscle

(ix) FEATURE:

- (A) NAME/KEY: sig_peptide
- (B) LOCATION: 215..292
- (C) IDENTIFICATION METHOD: Von Heijne matrix
- (D) OTHER INFORMATION: score 7
seq EMFLVLLVTGVHS/NK

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 72:

```
AAAAAGTACT GAGAGGTTGA TGGGACTGTT CGATTAGCTC CTCTGAGAAG AAGAGAAAAG   60
GTTCTTGGAC CTCTCCCTGT TTCTTCCTTA GAATAATTTG GATGGGATTT GTGATGCAGA   120
AAAGCCTAAG GGAAAAAGAA TATTCATTCT GTGTGGTGAA AATTTTTTGA AAAAAAATT   180
GCCTTCTTCA AACAAGGGTG TCATTCTGAT ATTT ATG AGG ACT GTT GTT CTC ACT   235
                               Met Arg Thr Val Val Leu Thr
                               -25                               -20

ATG AAG GCA TCT GTT ATT GAA ATG TTC CTT GTT TTG CTG GTG ACT GGA   283
Met Lys Ala Ser Val Ile Glu Met Phe Leu Val Leu Leu Val Thr Gly
          -15                               -10                               -5

GTA CAT TCA AAC AAA GAA ACG GCA AAG AAG ATT AAA AGG CCC GGG   328
Val His Ser Asn Lys Glu Thr Ala Lys Lys Ile Lys Arg Pro Gly
          1                               5                               10
```

(2) INFORMATION FOR SEQ ID NO: 73:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 281 base pairs
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: DOUBLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo Sapiens
- (D) DEVELOPMENTAL STAGE: Fetal
- (F) TISSUE TYPE: kidney

(ix) FEATURE:

- (A) NAME/KEY: sig_peptide
- (B) LOCATION: 150..269
- (C) IDENTIFICATION METHOD: Von Heijne matrix
- (D) OTHER INFORMATION: score 6.9
seq ISLLFIFFSIANS/SP

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 73:

```

ATTCTTTCCT TCTCATATCT ACAATTGCTC CTTTCTAGTT CAGTTCCTA GTACAGCTGG      60
AGTGATTATT KSKSKTTAAA AAATGCAAGC ATAAAAAGA AATAACAAA TAGTTAAATC      120
ATGTTATTCT TTTGTTTACA CTGTAATGA ATG TCT TCC CCA TTG CTT GTA GAA      173
                               Met Ser Ser Pro Leu Leu Val Glu
                               -40                               -35
CAA AGT TCT ACA AAG TCT CCC AAA AGC TGG TCC TGG TCC TTT CTA GCT      221
Gln Ser Ser Thr Lys Ser Pro Lys Ser Trp Ser Trp Ser Phe Leu Ala
      -30                               -25                               -20
TTC TCT TGC ATA AGT CTT CTT TTT ATT TTT TTC AGC ATT GCA AAT TCT      269
Phe Ser Cys Ile Ser Leu Leu Phe Ile Phe Phe Ser Ile Ala Asn Ser
      -15                               -10                               -5
TCC CCC TGC GGG
Ser Pro Cys Gly
1

```

(2) INFORMATION FOR SEQ ID NO: 74:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 179 base pairs
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: DOUBLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo Sapiens
- (D) DEVELOPMENTAL STAGE: Fetal
- (F) TISSUE TYPE: kidney

(ix) FEATURE:

- (A) NAME/KEY: sig_peptide
- (B) LOCATION: 96..170
- (C) IDENTIFICATION METHOD: Von Heijne matrix
- (D) OTHER INFORMATION: score 6.9
seq IPLLLLFFHLSFL/NS

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 74:

```

AGAACAAAGT TTAGAATGAT ATGTTTATGC CTGTGAACAT TTATCTTGTT AGATTATGCT      60
CACTAAGCCA TTGGGGTGTT TGGGGAATTT GATCA ATG TAT CTT TTC TGT CTC      113

```

Met Tyr Leu Phe Cys Leu
-25 -20

TTT TCA GTT TCG AAA ACT ATC CCT CTG CTG CTG CTT TTC TTC CAC TTG 161
Phe Ser Val Ser Lys Thr Ile Pro Leu Leu Leu Leu Phe Phe His Leu
-15 -10 -5

TCT TTT CTC AAT AGC TTG 179
Ser Phe Leu Asn Ser Leu
1

(2) INFORMATION FOR SEQ ID NO: 75:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 298 base pairs
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: DOUBLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo Sapiens
- (D) DEVELOPMENTAL STAGE: Fetal
- (F) TISSUE TYPE: kidney

(ix) FEATURE:

- (A) NAME/KEY: sig_peptide
- (B) LOCATION: 170..217
- (C) IDENTIFICATION METHOD: Von Heijne matrix
- (D) OTHER INFORMATION: score 6.9
seq CLLILKFLSPAET/SI

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 75:

ACAGAGTTCA CTTCTAGGAT ATTCCTTCCC AATCTTCACA GTCACCTCAT AGTCACTATG 60
AGGATTACAT GAGTKAATAT TTGTAAAAAG CGTTCAGGAG AGTGCTTGCT TCACATCAAA 120
TACTATATAT ACTTGTTAAA TAAATAGATC TCATTCACCC CACGAAACA ATG ATC GTT 178
Met Ile Val
-15
TGT CTC CTG ATT CTC AAG TTT TTG TCT CCA GCA GAG ACB TCT ATT CTG 226
Cys Leu Leu Ile Leu Lys Phe Leu Ser Pro Ala Glu Thr Ser Ile Leu
-10 -5 1
AGC TCC ATA GCT ACA TAT GGG GCT TTT TAT TTC ATA GTT CCA CTG GAG 274
Ser Ser Ile Ala Thr Tyr Gly Ala Phe Tyr Phe Ile Val Pro Leu Glu
5 10 15
GTT TCA CAA ATC CTT CAA ACT CAG 298
Val Ser Gln Ile Leu Gln Thr Gln
20 25

(2) INFORMATION FOR SEQ ID NO: 76:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 275 base pairs
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: DOUBLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo Sapiens
- (D) DEVELOPMENTAL STAGE: Fetal
- (F) TISSUE TYPE: kidney

(ix) FEATURE:

- (A) NAME/KEY: sig_peptide
- (B) LOCATION: 180..254
- (C) IDENTIFICATION METHOD: Von Heijne matrix
- (D) OTHER INFORMATION: score 6.7
seq LILCFLFILHTHT/HT

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 76:

```

ACAAACTGGT TACCCTGCCA CATGTATACC CCCTTCTCCC CATTCTCACT TCCTCGTTAG    60
ACGAAATGAT CATCCAGTGA AGCCATAGAT TATATTGGCC ATCTAATATC AAACCATATT    120
GGTCTCATTT GAAAATCTTT CATGATGCTT TGTGGTATTC ACAGTGAAGT TTAGATTCC    179
ATG GAT AAG AGC ATC AAG TCC TCT ATA ATC TGG TCT CTG ATT CTC TGT    227
Met Asp Lys Ser Ile Lys Ser Ser Ile Ile Trp Ser Leu Ile Leu Cys
-25                -20                -15                -10

TTT CTT TTT ATC CTG CAC ACA CAC ACA CAC ACA CAC ACA CAC ACA CAC    275
Phe Leu Phe Ile Leu His Thr His Thr His Thr His Thr His Thr His
      -5                1                5

```

(2) INFORMATION FOR SEQ ID NO: 77:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 405 base pairs
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: DOUBLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo Sapiens
- (D) DEVELOPMENTAL STAGE: Fetal
- (F) TISSUE TYPE: kidney

(ix) FEATURE:

- (A) NAME/KEY: sig_peptide
- (B) LOCATION: 283..390
- (C) IDENTIFICATION METHOD: Von Heijne matrix
- (D) OTHER INFORMATION: score 6.7
seq IFDLLLLLXXSNQ/LP

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 77:

```

ACAGACCTCT TTGAAATCT AATGAGAGCC ATAGACTTCA CCCTAAAAAA ATATATATGC      60
ATAAAAAGTT TAAATATAGT TTGGAGAGTA ACGCACCTTC CCCTAAAGCA ATTCCTAAAC    120
CTCATTTAAA GGATCTATAT TCTATAGTTC AGTTCTGCAT TTTAATGTC TTCTATATTG    180
TCTCATGCTA GAATAGTCAT TATATCTTCA TATGTAATAT TTAAAGTGTG AATTATCATC    240
TAACACTTCC TGTCTTCTGT CCCCCAAATC TATACTTCTC CC ATG TTC TTT ATT      294
                                   Met Phe Phe Ile
                                   -35

TTC ATT AAT GGC TTT ACW CTC CTT CTA ATG ACC CTA GCC ATG AAA CCC      342
Phe Ile Asn Gly Phe Thr Leu Leu Leu Met Thr Leu Ala Met Lys Pro
      -30                    -25                    -20

AGG CAT CCT ATT TTT GAC CTC TTG CTA TTG CTK RAB HTA TCT AAT CAA      390
Arg His Pro Ile Phe Asp Leu Leu Leu Leu Leu Xaa Xaa Ser Asn Gln
      -15                    -10                    -5

TTG CCA GTT ACG GGG
Leu Pro Val Thr Gly
  1              5
                                           405

```

(2) INFORMATION FOR SEQ ID NO: 78:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 215 base pairs
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: DOUBLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo Sapiens
- (D) DEVELOPMENTAL STAGE: Fetal
- (F) TISSUE TYPE: kidney

(ix) FEATURE:

- (A) NAME/KEY: sig_peptide
- (B) LOCATION: 3..182
- (C) IDENTIFICATION METHOD: Von Heijne matrix
- (D) OTHER INFORMATION: score 6.7
seq LWPFLTWINPALS/IC

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 78:

```

AC ATG TGC CCT AGT CTG GAA GAG GCT CCC AGT GTC AAG GGG ACT CTG      47
Met Cys Pro Ser Leu Glu Glu Ala Pro Ser Val Lys Gly Thr Leu
      -60                    -55                    -50

CCC TGC TCA GGA CAA CAG CAG CCT TTC CCG TTT GGA GCC TCA AAC ATC      95
Pro Cys Ser Gly Gln Gln Gln Pro Phe Pro Phe Gly Ala Ser Asn Ile

```

-45	-40	-35	-30	
CCA CTA CTC CTG GGC AGG AGC AGA AAG GTG GCT CGA GGT GCA CCG GTC	143			
Pro Leu Leu Leu Gly Arg Ser Arg Lys Val Ala Arg Gly Ala Pro Val				
-25	-20	-15		
CTG TGG CCA TTT CTC ACT TGG ATA AAC CCT GCA CTG TCC ATC TGT GAC	191			
Leu Trp Pro Phe Leu Thr Trp Ile Asn Pro Ala Leu Ser Ile Cys Asp				
-10	-5	1		
CCC TTA GGA TCC TGC GGA TGG CAG	215			
Pro Leu Gly Ser Cys Gly Trp Gln				
5	10			

(2) INFORMATION FOR SEQ ID NO: 79:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 400 base pairs
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: DOUBLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo Sapiens
- (D) DEVELOPMENTAL STAGE: Fetal
- (F) TISSUE TYPE: kidney

(ix) FEATURE:

- (A) NAME/KEY: sig_peptide
- (B) LOCATION: 287..337
- (C) IDENTIFICATION METHOD: Von Heijne matrix
- (D) OTHER INFORMATION: score 6.6
seq LLSALWFCHPCCL/CC

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 79:

AAGCTCCAAG GCAGGAAGAG AATTGGGCAT CGGGTACGAA CCTGGCAGCT CAGGAGTCGG	60
GGCTCCACTC ACCCCACACA AAAAGATGAA AAAAGCGCAW AGAGCTCAAT GCATTGATTG	120
GTTTGGCTGG GGACAGCCGG AGAAAGAAGC CCAAGAAAGG CCCAAGCAGT CACCGCCTGC	180
TTCGCACTGA GCCTCCCGAC TCATACTCTG AGTCCAGCTC CGAAGAGGAA GAGGAATTCTG	240
GTGTGGTTGG AAATCGCTCT CGCTTTGCCA AGGGAGACTA TTTACG ATG CTG CAA	295
Met Leu Gln	
-15	
GAT CTG TTA TCC GCT CTG TGG TTT TGT CAT CCT TGC TGC CTG TGT TGT	343
Asp Leu Leu Ser Ala Leu Trp Phe Cys His Pro Cys Cys Leu Cys Cys	
-10	-5
1	
GGC CTG TGT TGG CTT GGT GTG GAT GCA GGT TGC TCT CAA GGA GGA TCT	391
Gly Leu Cys Trp Leu Gly Val Asp Ala Gly Cys Ser Gln Gly Gly Ser	
5	10
15	

GGA TGC CCG
Gly Cys Pro
20

400

(2) INFORMATION FOR SEQ ID NO: 80:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 340 base pairs
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: DOUBLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo Sapiens
- (D) DEVELOPMENTAL STAGE: Fetal
- (F) TISSUE TYPE: kidney

(ix) FEATURE:

- (A) NAME/KEY: sig_peptide
- (B) LOCATION: 167..223
- (C) IDENTIFICATION METHOD: Von Heijne matrix
- (D) OTHER INFORMATION: score 6.6
seq LLSLAAYLSGPHQ/EP

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 80:

```

AAAATGTCCT CCACAGCTTT GCCCAGTGGG ACACATGGCT CCTGACATAC GTAACCCAGG    60
ATGGGATGCC TTGTTGGAGT CTCTCAGATA TGGAGCAAAA TGGGCCATGT GCAGTCAAGA    120
CGCCATCTAM CCTGGGCAGC TTGCCTAAGC CTCGAGGGAC CTGCCA ATG ATG GAT      175
                                   Met Met Asp
CTG AGA CCT CTT CTG TCC CTG GCT GCC TAT CTG TCT GGT CCT CAT CAA      223
Leu Arg Pro Leu Leu Ser Leu Ala Ala Tyr Leu Ser Gly Pro His Gln
-15                               -10                               -5
GAA CCC AGT GTT CCC ACC CGA GAT GGA GAC GTG AAT AAT CTT CCT AAG      271
Glu Pro Ser Val Pro Thr Arg Asp Gly Asp Val Asn Asn Leu Pro Lys
1                               5                               10                               15
CCT AAT CCT GCC AGA AGC GTG AAG CAA GGG GGA ATH TGG AAG GCG GAA      319
Pro Asn Pro Ala Arg Ser Val Lys Gln Gly Gly Ile Trp Lys Ala Glu
20                               25                               30
CAG GAA AGA GTG GAA GTG GAG
Gln Glu Arg Val Glu Val Glu
35

```

340

(2) INFORMATION FOR SEQ ID NO: 81:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 245 base pairs
- (B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: DOUBLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo Sapiens
(F) TISSUE TYPE: Heart

(ix) FEATURE:

(A) NAME/KEY: sig_peptide
(B) LOCATION: 147..203
(C) IDENTIFICATION METHOD: Von Heijne matrix
(D) OTHER INFORMATION: score 6.6
seq LLPGLPLVRTSFS/HF

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 81:

```
AGCGGTCAGA GGATGCCCTC TTCGCCCTGT GAGCAGCTCT GTGGTTTGCC TCCCCAGATG    60
GCGGGTCCCC GCTTGACACC CGTGGACACC GGGCACTGGC CACTCCTACA TCCCCAGCTC   120
CACACGGCCT GCACACCTGT GTTTCC ATG GAA ATG CCA CCG TGT CTG CTC CCA    173
                               Met Glu Met Pro Pro Cys Leu Leu Pro
                               -15

GGC CTC CCA CTA GTC AGG ACC AGC TTC AGC CAC TTC TTT TCT CTG AGT    221
Gly Leu Pro Leu Val Arg Thr Ser Phe Ser His Phe Phe Ser Leu Ser
-10                      -5                      1                      5

GGT GGG ACA ACT ACA GCC AGA GGG                                245
Gly Gly Thr Thr Thr Ala Arg Gly
                        10
```

(2) INFORMATION FOR SEQ ID NO: 82:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 192 base pairs
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: DOUBLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo Sapiens
(F) TISSUE TYPE: Muscle

(ix) FEATURE:

(A) NAME/KEY: sig_peptide
(B) LOCATION: 19..93
(C) IDENTIFICATION METHOD: Von Heijne matrix
(D) OTHER INFORMATION: score 6.5
seq GLAMLHVTRGVXG/SR

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 82:

ACATGCGCAG GAGGCTCA ATG ACA GTC GAG CTT TGG CTA AGG CTC CGG GGA	51
Met Thr Val Glu Leu Trp Leu Arg Leu Arg Gly	
-25 -20 -15	
AAG GGT CTA GCC ATG CTG CAT GTG ACC CGG GGG GTC TRG GGG TCC AGG	99
Lys Gly Leu Ala Met Leu His Val Thr Arg Gly Val Xaa Gly Ser Arg	
-10 -5 1	
GTC CGA GTA TRG YCA MTG TTG CCC GCG CTC CTC GGG MCC CCC MGG GCC	147
Val Arg Val Xaa Xaa Xaa Leu Pro Ala Leu Leu Gly Xaa Pro Arg Ala	
5 10 15	
CTC TCA TCG MTG GCA GCC AAA ATG GGG GAK TAT CGC AAS ATG TGG	192
Leu Ser Ser Xaa Ala Ala Lys Met Gly Xaa Tyr Arg Xaa Met Trp	
20 25 30	

(2) INFORMATION FOR SEQ ID NO: 83:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 126 base pairs
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: DOUBLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo Sapiens
- (F) TISSUE TYPE: Dystrophic muscle

(ix) FEATURE:

- (A) NAME/KEY: sig_peptide
- (B) LOCATION: 7..78
- (C) IDENTIFICATION METHOD: Von Heijne matrix
- (D) OTHER INFORMATION: score 6.4
seq LLILLCSSPPDRV/SY

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 83:

ACAAAC ATG TCT ATA GAA GAT TTT GTG AAT AGA AGC ATA CTT CTG ATC	48
Met Ser Ile Glu Asp Phe Val Asn Arg Ser Ile Leu Leu Ile	
-20 -15	
TTG CTC TGT TCT TCC CCA CCT GAT AGG GTC AGC TAC AGA GCC AAG GTT	96
Leu Leu Cys Ser Ser Pro Pro Asp Arg Val Ser Tyr Arg Ala Lys Val	
-10 -5 1 5	
TTA CAC TCA TTG CTT CAA TTG CCC GCC CAG	126
Leu His Ser Leu Leu Gln Leu Pro Ala Gln	
10 15	

(2) INFORMATION FOR SEQ ID NO: 84:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 184 base pairs
- (B) TYPE: NUCLEIC ACID

- (C) STRANDEDNESS: DOUBLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo Sapiens
- (F) TISSUE TYPE: Muscle

(ix) FEATURE:

- (A) NAME/KEY: sig_peptide
- (B) LOCATION: 32..91
- (C) IDENTIFICATION METHOD: Von Heijne matrix
- (D) OTHER INFORMATION: score 6.4
seq FALLFLFLVPVPG/HG

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 84:

```

AAGTCTCAGC GTGGGGTGAA GCCTAGCAGC T ATG AGG ATC CAT TAT CTT CTG      52
                               Met Arg Ile His Tyr Leu Leu
                               -20                      -15

TTT GCT TTG CTC TTC CTG TTT TTG GTG CCT GTT CCA GGT CAT GGA GGA      100
Phe Ala Leu Leu Phe Leu Phe Leu Val Pro Val Pro Gly His Gly Gly
                    -10                      -5                      1

ATC ATA AAC ACA TTA CAG AAA TAT TAA TTG CAG AGT CAG AGG CGG CCG      148
Ile Ile Asn Thr Leu Gln Lys Tyr Xaa Leu Gln Ser Gln Arg Arg Pro
                    5                      10                      15

GTG TGC TGT GCT CAG CTG CCT TCC AAA GGA GAA AGG                      184
Val Cys Cys Ala Gln Leu Pro Ser Lys Gly Glu Arg
    20                      25                      30

```

(2) INFORMATION FOR SEQ ID NO: 85:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 375 base pairs
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: DOUBLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo Sapiens
- (D) DEVELOPMENTAL STAGE: Fetal
- (F) TISSUE TYPE: kidney

(ix) FEATURE:

- (A) NAME/KEY: sig_peptide
- (B) LOCATION: 217..255
- (C) IDENTIFICATION METHOD: Von Heijne matrix
- (D) OTHER INFORMATION: score 6.4
seq MCLLTALVTQVIS/LR

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 85:

ATCTGGCGCG	TGGTCTTGCA	TTTCTACTT	GGTCCTGTTC	GTGGCGCCGC	GCCTCCGGGT	60									
GTTGGGGAGT	CCGGG	ATG	ATG	GGG	AAT	CCG	GGG	CTC	GCC	CTA	GTC	GCG	GGG	111	
	Met	Met	Gly	Asn	Pro	Gly	Leu	Ala	Leu	Val	Ala	Gly			
				-15						-10					
ACA	CCG	CCT	TCC	AGG	AGC	TGT	CCC	CAG	GCA	AAC	TCA	CAG	ACG	CGG	156
Thr	Pro	Pro	Ser	Arg	Ser	Cys	Pro	Gln	Ala	Asn	Ser	Gln	Thr	Arg	
	-5					1				5					

(2) INFORMATION FOR SEQ ID NO: 87:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 458 base pairs
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: DOUBLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo Sapiens
- (D) DEVELOPMENTAL STAGE: Fetal
- (F) TISSUE TYPE: kidney

(ix) FEATURE:

- (A) NAME/KEY: sig_peptide
- (B) LOCATION: 186..299
- (C) IDENTIFICATION METHOD: Von Heijne matrix
- (D) OTHER INFORMATION: score 6.3
seq PCVSLWAPRXFA/SS

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 87:

```

ATAACCCATA TAGTAGTTAA GCCATTGTGG TGAGGGTGTT TGAAACCCAG CTATCCTATG      60
TAATGCTATT TCCAGGGGAA AAATATTCCC AATTCCAGGT AAAAGATCAG AAACAGATAT      120
CACCTGSAWT TTGTTCCACC TTCACCCCAG GCTTCAGCTA TACTTAGGTA TTA CTCTCTG      180
GTCCC ATG AAC CAT CTC ATG CCT TTG ACT GTG CTG CAC TCA GTG CTT GAA      230
Met Asn His Leu Met Pro Leu Thr Val Leu His Ser Val Leu Glu
      -35                      -30                      -25

ATG CTC CGC ACA CCC CGC ACA CCT CCC TGG CCC TGT GTA TCC CTT CTA      278
Met Leu Arg Thr Pro Arg Thr Pro Pro Trp Pro Cys Val Ser Leu Leu
      -20                      -15                      -10

TGG GCG CCC AGA GSA TTT GCT TCC TCT TGC TCT CAA GCA TTT ACC ACT      326
Trp Ala Pro Arg Xaa Phe Ala Ser Ser Cys Ser Gln Ala Phe Thr Thr
      -5                      1                      5

CTG CAN KGC AAT TGC TTG CTT ACT AAT CCA TCT CCC ACA CTA GAT TGT      374
Leu Xaa Xaa Asn Cys Leu Leu Thr Asn Pro Ser Pro Thr Leu Asp Cys
      10                      15                      20                      25

GAC CTC CCT GAG GGC TCA GAA ATA TTA AAT TCT TCT CTG TAT CCT CAT      422
Asp Leu Pro Glu Gly Ser Glu Ile Leu Asn Ser Ser Leu Tyr Pro His
      30                      35                      40

TGC CTA CTC AGT GCT TGG AAC ACA CGA CAC TCA ACA      458
Cys Leu Leu Ser Ala Trp Asn Thr Arg His Ser Thr
      45                      50

```

(2) INFORMATION FOR SEQ ID NO: 88:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 138 base pairs

- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: DOUBLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo Sapiens
- (D) DEVELOPMENTAL STAGE: Fetal
- (F) TISSUE TYPE: kidney

(ix) FEATURE:

- (A) NAME/KEY: sig_peptide
- (B) LOCATION: 13..84
- (C) IDENTIFICATION METHOD: Von Heijne matrix
- (D) OTHER INFORMATION: score 6.3
seq SLLXLRASQLSEG/DT

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 88:

```

ATTATTATTT TT ATG GGA CAT GTT GTG TTT GGG GAT ATA AAA AAT AGT TTA    51
      Met Gly His Val Val Phe Gly Asp Ile Lys Asn Ser Leu
                        -20                               -15

TTA KGT TTA AGG GCT TCG CAG CTT AGT GAG GGA GAC ACA TGR VTG AAM    99
Leu Xaa Leu Arg Ala Ser Gln Leu Ser Glu Gly Asp Thr Xaa Xaa Xaa
-10                        -5                               1                               5

TVA TGT CCA BRT ATG RTG AGA GGT AAA CAC ATA TCC TAT
Xaa Cys Pro Xaa Met Xaa Arg Gly Lys His Ile Ser Tyr    138
      10                        15

```

(2) INFORMATION FOR SEQ ID NO: 89:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 341 base pairs
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: DOUBLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo Sapiens
- (F) TISSUE TYPE: Heart

(ix) FEATURE:

- (A) NAME/KEY: sig_peptide
- (B) LOCATION: 48..290
- (C) IDENTIFICATION METHOD: Von Heijne matrix
- (D) OTHER INFORMATION: score 6.3
seq FLSLLXSVSETPG/SL

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 89:

```

ACTTCTTCCC CGGGTCTCCG AAGCCGCTAG GGAAGCGCAA GTGGGCC ATG GCT GGC    56
                        Met Ala Gly

```

-80

GGG AGG CGG GAT TAC AGC CAG CTC TTT GGC CGC GGC CCC GGT CGG CTC	104
Gly Arg Arg Asp Tyr Ser Gln Leu Phe Gly Arg Gly Pro Gly Arg Leu	
-75 -70 -65	
TCG CGA GCG CGA GCC TCT GTT GTG CGT TGG TCT CCC CGG GCA ACT GCT	152
Ser Arg Ala Arg Ala Ser Val Val Arg Trp Ser Pro Arg Ala Thr Ala	
-60 -55 -50	
TGC CCT GCG CCA CCG AGC CTC CCG GAT TTA AAG CGG CAG GAG CTG GTT	200
Cys Pro Ala Pro Pro Ser Leu Pro Asp Leu Lys Arg Gln Glu Leu Val	
-45 -40 -35	
AGC CGG ATA GAA TGT GGG TGC CGA GGG CCG GTG GGG GCC ACC GCA GAC	248
Ser Arg Ile Glu Cys Gly Cys Arg Gly Pro Val Gly Ala Thr Ala Asp	
-30 -25 -20 -15	
TTC TTT CTG TCC CTG CTC TDC AGC GTC TCT GAA ACC CCT GGC AGC CTG	296
Phe Phe Leu Ser Leu Leu Xaa Ser Val Ser Glu Thr Pro Gly Ser Leu	
-10 -5 1	
CGG RGA AAC GAT CTT TTC TTC GTC TCT CAG CTT ATT TGG GGC CGG	341
Arg Xaa Asn Asp Leu Phe Phe Val Ser Gln Leu Ile Trp Gly Arg	
5 10 15	

(2) INFORMATION FOR SEQ ID NO: 90:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 272 base pairs
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: DOUBLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo Sapiens
- (D) DEVELOPMENTAL STAGE: Fetal
- (F) TISSUE TYPE: kidney

(ix) FEATURE:

- (A) NAME/KEY: sig_peptide
- (B) LOCATION: 207..263
- (C) IDENTIFICATION METHOD: Von Heijne matrix
- (D) OTHER INFORMATION: score 6.1
seq LWCFHSFISFSL/SS

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 90:

ATCCTCCATA GCTATATCCA TTTCCTGGGA CATGGGTTGG CCCAAGAGGG AATGAGAAGG	60
ACCTGCGATT GCACAGGAAA TTCTGGGGCA CATTTAACGT TAAATCATTAGCTTCTGCC	120
AATAAATCCA TTAAGTTTAA TTAACTGAG ATGGCCAACG ATCTGCTGAC AATATTCCTT	180
CATTGATTTT CATTCTCAGT GAATCG ATG TTC TGG CNT GGC TCT CTT TGG TGT	233
Met Phe Trp Xaa Gly Ser Leu Trp Cys	

-15

TTT CAT TCT TTC ATT TCT TTC TCC CTG TCC TCA TCA CGG
 Phe His Ser Phe Ile Ser Phe Ser Leu Ser Ser Ser Arg
 -10 -5 1

272

(2) INFORMATION FOR SEQ ID NO: 91:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 351 base pairs
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: DOUBLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo Sapiens
- (D) DEVELOPMENTAL STAGE: Fetal
- (F) TISSUE TYPE: kidney

(ix) FEATURE:

- (A) NAME/KEY: sig_peptide
- (B) LOCATION: 118..225
- (C) IDENTIFICATION METHOD: Von Heijne matrix
- (D) OTHER INFORMATION: score 6
seq FLLTFFSYSLHA/SR

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 91:

AGGCNNNCGG ASCSGGGCTG GAGAGCGGCS NCCACTGCGG ATCTCGGAAG GAAGAAATGA 60
 TGTAATCAC TCATSSAVAC TTTAAGGTCN NNGTGAGAM GGAAGGTCAG GMAGAAC 117
 ATG GCC TGG CCA AAT GTT TTT CAA ABA GGG TCT CTG CTG TCC CAG TTC 165
 Met Ala Trp Pro Asn Val Phe Gln Xaa Gly Ser Leu Leu Ser Gln Phe
 -35 -30 -25
 AKN BAT CAT CAT GTT GTA GTG TTC CTG CTC ACT TTC TTC AGT TAT TCG 213
 Xaa Xaa His His Val Val Val Phe Leu Leu Thr Phe Phe Ser Tyr Ser
 -20 -15 -10 -5
 TTG CTC CAT GCT TCA CGA AAA ACA TTT RGC AAT GTC AAA GTC AGT ATC 261
 Leu Leu His Ala Ser Arg Lys Thr Phe Xaa Asn Val Lys Val Ser Ile
 1 5 10
 TCT GAG CAG TGG ACC CCA AGT GCT TTT AAC ACG TCA GTT GAG CTG CCT 309
 Ser Glu Gln Trp Thr Pro Ser Ala Phe Asn Thr Ser Val Glu Leu Pro
 15 20 25
 GTG GAG ATC TGG AGC AGC RAC CAT TTG TTC CCC AGT GCA GAG 351
 Val Glu Ile Trp Ser Ser Xaa His Leu Phe Pro Ser Ala Glu
 30 35 40

(2) INFORMATION FOR SEQ ID NO: 92:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 466 base pairs
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: DOUBLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo Sapiens
- (D) DEVELOPMENTAL STAGE: Fetal
- (F) TISSUE TYPE: kidney

(ix) FEATURE:

- (A) NAME/KEY: sig_peptide
- (B) LOCATION: 380..436
- (C) IDENTIFICATION METHOD: Von Heijne matrix
- (D) OTHER INFORMATION: score 6
seq WILAVGLSLPSSS/XI

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 92:

```

ACTCTCTTCT ACTGGAATGG TACCCTTGTT GACTGACTCA TGTATAGCTG CTTGGCTTAA   60
TGGTAGACCA GATATTCAGG TCCTCTGAGA CAGGCCCTG ATGACTTTTG CAACTACATC  120
TTTCAMCACA GCCTGCCTTG CATTTTGGAC TCTAGCAACA CTGAAATACA TGTCATTTCC  180
CAAGGCATGT TAAGCTGTTT CTATTCTCTA GGCTCTCCCT TTTTCCTAGA ATGCCCTTTT  240
CCTCTAGGCT AATGTCTTTC TCCTTTAAAT TAGTCATCTT CAACAAAGGC TACCTTGACC  300
TTCTCTTGAC TTTGCCACAT TCCTGCTGCT GCCTTCCTTC CATGGCCTTT GTCACGCTAT  360
ATGGTAATTG ACAGGTTCC ATG ATC TTG AGG AAC TTA TGG ATT TTA GCA GTG   412
                Met Ile Leu Arg Asn Leu Trp Ile Leu Ala Val
                -15                               -10

GGT CTT AGC TTG CCA TCT TCT TCA MCC ATC AAG TTT CAT TTC TCT CTT   460
Gly Leu Ser Leu Pro Ser Ser Ser Xaa Ile Lys Phe His Phe Ser Leu
                -5                               1                               5

TAC TCA
Tyr Ser
    10

```

(2) INFORMATION FOR SEQ ID NO: 93:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 389 base pairs
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: DOUBLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo Sapiens

(D) DEVELOPMENTAL STAGE: Fetal
(F) TISSUE TYPE: kidney

(ix) FEATURE:

(A) NAME/KEY: sig_peptide
(B) LOCATION: 267..371
(C) IDENTIFICATION METHOD: Von Heijne matrix
(D) OTHER INFORMATION: score 5.9
seq LCGLLHLWLKVFS/LK

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 93:

```

ACAATCAGTT TGCCAATACC TCAGAAACAA ATACCTCGGA CAAATCTTTC TCTAAAGACC   60
TCAGTCAGAT ACTAGTCAAT ATCAAATCAT GTAGATGGCG GCATTTTAGG CCTCGGACAC   120
CATCCCTACA TGACAGTGAC AATGATGAAC TCTCCTGTAG AAAATTATAT AGGAGTATAA   180
ACCGAACAGG AACAGCACAA CCTGGGACCC AGACATGCAG TACCTCTACG CAAAGTAAAA   240
GTAGCAGTGG TTCAGCACAC TTTGGT ATG TTG ACT GTT AAT GAT GTA CGT TTC   293
                        Met Leu Thr Val Asn Asp Val Arg Phe
                        -35                               -30

TAT AGA AAT GTC AGG TCC AAC CAT TTC CCA TTT GTT CGA CTA TGT GGT   341
Tyr Arg Asn Val Arg Ser Asn His Phe Pro Phe Val Arg Leu Cys Gly
-25                               -20                               -15

CTG TTA CAT TTA TGG CTT AAA GTC TTT TCT CTT AAA CAG TTA AAA AAA   389
Leu Leu His Leu Trp Leu Lys Val Phe Ser Leu Lys Gln Leu Lys Lys
-10                               -5                               1                               5

```

(2) INFORMATION FOR SEQ ID NO: 94:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 272 base pairs
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: DOUBLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo Sapiens
(D) DEVELOPMENTAL STAGE: Fetal
(F) TISSUE TYPE: kidney

(ix) FEATURE:

(A) NAME/KEY: sig_peptide
(B) LOCATION: 111..179
(C) IDENTIFICATION METHOD: Von Heijne matrix
(D) OTHER INFORMATION: score 5.9
seq LFLNLCILAXPFS/KQ

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 94:

```

ATTAATTTTA ATTTTCATTG TCAATATTTT GAGCTTAGAA CATTTATGGT ATAAAAATTT   60

```

```

AAACTAATCA AAGTTGTGTG ATGATTTC CG GGAATTATTA TTGAAAGCCT ATG AAT      116
                                   Met Asn

TTA AAA CCA GGT TTA CCA TGT AAT TTG TTT TTA AAT TTA TGT ATA CTA      164
Leu Lys Pro Gly Leu Pro Cys Asn Leu Phe Leu Asn Leu Cys Ile Leu
-20                               -15                               -10

GCC TGV CCT TTC TCC AAG CAA ATT ATT GAA CTA TTA GAA TAT GTT AGT      212
Ala Xaa Pro Phe Ser Lys Gln Ile Ile Glu Leu Leu Glu Tyr Val Ser
-5                               1                               5                               10

TAT CAT CCT TGT GTC TTA GTA TAT AGT GAA TAC AGM AAC ATC AGC ATT      260
Tyr His Pro Cys Val Leu Val Tyr Ser Glu Tyr Xaa Asn Ile Ser Ile
15                               20                               25

GTA TAC ACT CTT                                                         272
Val Tyr Thr Leu
30

```

(2) INFORMATION FOR SEQ ID NO: 95:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 345 base pairs
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: DOUBLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo Sapiens
- (D) DEVELOPMENTAL STAGE: Fetal
- (F) TISSUE TYPE: kidney

(ix) FEATURE:

- (A) NAME/KEY: sig_peptide
- (B) LOCATION: 43..162
- (C) IDENTIFICATION METHOD: Von Heijne matrix
- (D) OTHER INFORMATION: score 5.9
seq VVLAWGLLNVSMA/GM

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 95:

```

ACCAGAGAGA GTGGCGCGAG CTGCGTTTTTC CGGCCAGAGG AC ATG ATG CAG GGG      54
                                   Met Met Gln Gly
                                   -40

GAG GCA CAC CCT AGT GCT TCC CTT ATT GAC AGA ACC ATC AAG ATG AGA      102
Glu Ala His Pro Ser Ala Ser Leu Ile Asp Arg Thr Ile Lys Met Arg
-35                               -30                               -25

AAA GAA ACA GAG GCT AGG AAA GTG GTC TTA GCC TGG GGA CTC CTA AAT      150
Lys Glu Thr Glu Ala Arg Lys Val Val Leu Ala Trp Gly Leu Leu Asn
-20                               -15                               -10                               -5

GTA TCT ATG GCT GGA ATG ATA TAT ACT GAA ATG ACT GGA AAA TTG ATT      198
Val Ser Met Ala Gly Met Ile Tyr Thr Glu Met Thr Gly Lys Leu Ile

```

	1	5	10	
AGT TCA TAC TAC AAT GTG ACA TAC TGG CCC CTC TGG TAT ADY GAG CTT				246
Ser Ser Tyr Tyr Asn Val Thr Tyr Trp Pro Leu Trp Tyr Xaa Glu Leu	15	20	25	
GCC CTT GCA TCT CTC TTC AGC CTT AAT GCC TTA TTT GAT TTT TGG AGA				294
Ala Leu Ala Ser Leu Phe Ser Leu Asn Ala Leu Phe Asp Phe Trp Arg	30	35	40	
TAT TTC AAA TAT ACT GTG GCA CCA ACA AGT CTG GTT GTT AGT CCT GGA				342
Tyr Phe Lys Tyr Thr Val Ala Pro Thr Ser Leu Val Val Ser Pro Gly	45	50	55	60
CGG				
Arg				345

(2) INFORMATION FOR SEQ ID NO: 96:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 447 base pairs
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: DOUBLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo Sapiens
- (D) DEVELOPMENTAL STAGE: Fetal
- (F) TISSUE TYPE: kidney

(ix) FEATURE:

- (A) NAME/KEY: sig_peptide
- (B) LOCATION: 274..330
- (C) IDENTIFICATION METHOD: Von Heijne matrix
- (D) OTHER INFORMATION: score 5.9
seq PXXLLILAHITQS/CP

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 96:

AGTATTTGTT AAATGCTACA AGAGTGA CTG GGATCATAAG TGTTACGGGA GTTTGGCAAA	60
GAAGCAGGAG GTAGTTAGTG TAACTGTTAA TGTGATTATA AGACTAATAC ATTTTGTKGG	120
RAGATAACTT ACCAAGTTTG GTTTGTGGAA AATTGGATT GAGAAGGAAA TTGTATGTTT	180
CCGTTAGAAG TAGAACAACA ACAACAAAT ATCTCCCATC ATTTGTTTGG TACTATCTGG	240
CCTCCCCAGT GCTGCTTGGG AGAATCATGA AAC ATG ATG AAT CAA ACA CAT CCT	294
Met Met Asn Gln Thr His Pro	
-15	
TRM RTG TTG CTC ATC CTG GCA CAT ATT ACA CAG AGT TGC CCA TGG GCC	342
Xaa Xaa Leu Leu Ile Leu Ala His Ile Thr Gln Ser Cys Pro Trp Ala	
-10 -5 1	
CAT GTA GGA GCA GCT CCA TCT GCC CTT CTA ATA CAT AGG TGG GAR CTG	390

His Val Gly Ala Ala Pro Ser Ala Leu Leu Ile His Arg Trp Glu Leu
 5 10 15 20

AGG GGG TGC TCG TAT TTG AAA CTG TTT TTG GTT ATG GTG CTC ATA TTT 438
 Arg Gly Cys Ser Tyr Leu Lys Leu Phe Leu Val Met Val Leu Ile Phe
 25 30 35

GAA ATG CTT 447
 Glu Met Leu

(2) INFORMATION FOR SEQ ID NO: 97:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 355 base pairs
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: DOUBLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo Sapiens
- (D) DEVELOPMENTAL STAGE: Fetal
- (F) TISSUE TYPE: kidney

(ix) FEATURE:

- (A) NAME/KEY: sig_peptide
- (B) LOCATION: 35..94
- (C) IDENTIFICATION METHOD: Von Heijne matrix
- (D) OTHER INFORMATION: score 5.8
seq GLVLLLSLAEILF/KI

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 97:

AGTCCTAGTC AGAGTTTCT GTGAAGGCAA GGGC ATG GGG TTG CCG GAG AGA AGA 55
 Met Gly Leu Pro Glu Arg Arg
 -20 -15

GGA TTG GTC CTG CTT TTA AGC CTA GCT GAA ATT CTT TTC AAG ATC ATG 103
 Gly Leu Val Leu Leu Leu Ser Leu Ala Glu Ile Leu Phe Lys Ile Met
 -10 -5 1

ATT CTG GAA GGA GGT GGT GTA ATG AAT CTC AAC CCC GGC AAC AAC CTC 151
 Ile Leu Glu Gly Gly Gly Val Met Asn Leu Asn Pro Gly Asn Asn Leu
 5 10 15

CTT CAC CAG CCG CCA GCC TGG ACA GAC AGC TAC TCC ACG TGC AAT GTT 199
 Leu His Gln Pro Pro Ala Trp Thr Asp Ser Tyr Ser Thr Cys Asn Val
 20 25 30 35

TCC AGT GGG TTT TTT GGA GGC CAG TGG CAT GAA ATT CAT CCT CAG TAC 247
 Ser Ser Gly Phe Phe Gly Gly Gln Trp His Glu Ile His Pro Gln Tyr
 40 45 50

TGG ACC AAG TAC CAG GTG TGG GAG TGG CTC CAG CAC CTC CTG GAC ACC 295
 Trp Thr Lys Tyr Gln Val Trp Glu Trp Leu Gln His Leu Leu Asp Thr
 55 60 65

AAC CAG CTG GAT GCC AAT TGT ATC CCT TTC CAA GAG TTC GAC ATC AAC 343
 Asn Gln Leu Asp Ala Asn Cys Ile Pro Phe Gln Glu Phe Asp Ile Asn
 70 75 80

GGC GAG CAM CGG
 Gly Glu Xaa Arg 355
 85

(2) INFORMATION FOR SEQ ID NO: 98:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 409 base pairs
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: DOUBLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo Sapiens
- (D) DEVELOPMENTAL STAGE: Fetal
- (F) TISSUE TYPE: kidney

(ix) FEATURE:

- (A) NAME/KEY: sig_peptide
- (B) LOCATION: 305..388
- (C) IDENTIFICATION METHOD: Von Heijne matrix
- (D) OTHER INFORMATION: score 5.8
seq LCWALLYNCFSSS/CV

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 98:

ATCAGTCTGT GGAGACAGGT GAGCACGAAC TTCTGAGACA GGTGTGGGTG CGAGGGTCGG 60
 GAGGGTCATG GGATTGGGAC CGAGGTGTGA GGAGGGAATC TGCAATTCCT TGCTACACAG 120
 AGCGCTGGCA ACTTCTGACA GGCTGTTTCT GGGGTATGGG CTGCCTCGGG TTGTTGCTGT 180
 TACAAGGAAA GAAAAGAGTT CCCCTGCCCA CCGCCTCCCA GCCACTGGGC TACCTCCTGG 240
 CAGGAAATTT GCAAACCTGAG TTAAACAAGT TAGGATCAGC AGAGGGTAGA GGAGGGCCTG 300
 GCAG ATG TGG GGT CTA GAA GAG GAC AGG AGT TAT CAG GGS CTC CGG CCA 349
 Met Trp Gly Leu Glu Glu Asp Arg Ser Tyr Gln Gly Leu Arg Pro
 -25 -20 -15

TTG TGC TGG GCT TTG CTG TAC AAT TGT TTC TCA AGC AGT TGT GTY CCT 397
 Leu Cys Trp Ala Leu Leu Tyr Asn Cys Phe Ser Ser Ser Cys Val Pro
 -10 -5 1

GTG GCT TTG GTG
 Val Ala Leu Val 409
 5

(2) INFORMATION FOR SEQ ID NO: 99:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 401 base pairs
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: DOUBLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo Sapiens
- (D) DEVELOPMENTAL STAGE: Fetal
- (F) TISSUE TYPE: kidney

(ix) FEATURE:

- (A) NAME/KEY: sig_peptide
- (B) LOCATION: 129..383
- (C) IDENTIFICATION METHOD: Von Heijne matrix
- (D) OTHER INFORMATION: score 5.7
seq ALLASLGIAFSRS/RA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 99:

```

AGTAGCGGAC ATTTTGTTC TGTCAGGCTG TCCCTGGCCG GGGTTCTGTA ACGCTTGTGT    60
GGGCCGCAGG TGGAGGTGTT GGGAAAGCGC GGAGGAGATG TTGTCCCCAG TGTCCCGAGA    120
CGCGTCTG ATG CTC TGC AGG GAC GGA AGT GCC TGC GTC CCC CGA TCG AGA    170
    Met Leu Cys Arg Asp Gly Ser Ala Cys Val Pro Arg Ser Arg
    -85                -80                -75

CGC CTG CCG CTC CCG GCA GCT GTC CGC GCC CAC GGT CCT ATG GCG GAC    218
Arg Leu Pro Leu Pro Ala Ala Val Arg Ala His Gly Pro Met Ala Asp
    -70                -65                -60

TGN NCG GAC TCC GCG CGG GGC TGT GTG GTC TTT GAG GAT GTG TTT GTA    266
Xaa Xaa Asp Ser Ala Arg Gly Cys Val Val Phe Glu Asp Val Phe Val
    -55                -50                -45                -40

TAC TTC TCT CGG GAA GAA TGG GAG CTT CTT GAT GAT GCT CAG AGA CTT    314
Tyr Phe Ser Arg Glu Glu Trp Glu Leu Leu Asp Asp Ala Gln Arg Leu
    -35                -30                -25

TTG TAC CAT GAT GTG ATG CTG GAG AAC TTT GCA CTT TTA GCC TCA CTG    362
Leu Tyr His Asp Val Met Leu Glu Asn Phe Ala Leu Leu Ala Ser Leu
    -20                -15                -10

GGA ATT GCA TTT TCC AGA TCA CGT GCA GTC ATG AAA CTA    401
Gly Ile Ala Phe Ser Arg Ser Arg Ala Val Met Lys Leu
    -5                1                5

```

(2) INFORMATION FOR SEQ ID NO: 100:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 261 base pairs
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: DOUBLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo Sapiens
- (D) DEVELOPMENTAL STAGE: Fetal
- (F) TISSUE TYPE: kidney

(ix) FEATURE:

- (A) NAME/KEY: sig_peptide
- (B) LOCATION: 61..228
- (C) IDENTIFICATION METHOD: Von Heijne matrix
- (D) OTHER INFORMATION: score 5.7
seq FLCFLNLTSHLSG/LD

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 100:

ATACCTAATG ATAACACAGT ATCTCTTCGA ATTTGTACTA TTGCAGAACA TTTAGAAACA	60
ATG CTT ATT ACT CGK TTA CAG TCT GGT ATA GAT TTT GCA ATC CAG CTT	108
Met Leu Ile Thr Arg Leu Gln Ser Gly Ile Asp Phe Ala Ile Gln Leu	
-55 -50 -45	
GAT GAA AGC ACT GAT ATT GGA AGC TGC ACA ACA CTT TTA GTT TAT GTC	156
Asp Glu Ser Thr Asp Ile Gly Ser Cys Thr Thr Leu Leu Val Tyr Val	
-40 -35 -30 -25	
AGA TAT GCG TGG CAA GAT GAT TTT TTG GAG GAT TTT TTG TGT TTT TTA	204
Arg Tyr Ala Trp Gln Asp Asp Phe Leu Glu Asp Phe Leu Cys Phe Leu	
-20 -15 -10	
AAT TTA ACC TCA CAC CTA AGT GGA TTA GAT ATT TTT ACA GAA TTA GAA	252
Asn Leu Thr Ser His Leu Ser Gly Leu Asp Ile Phe Thr Glu Leu Glu	
-5 1 5	
AGG CGC GGG	
Arg Arg Gly	261
10	

(2) INFORMATION FOR SEQ ID NO: 101:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 382 base pairs
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: DOUBLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo Sapiens
- (D) DEVELOPMENTAL STAGE: Fetal
- (F) TISSUE TYPE: kidney

(ix) FEATURE:

- (A) NAME/KEY: sig_peptide
- (B) LOCATION: 191..304
- (C) IDENTIFICATION METHOD: Von Heijne matrix
- (D) OTHER INFORMATION: score 5.7

seq LAFLSCLAFLVLD/TQ

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 101:

```

AACTCTGCAG GGCCTCCAAG GCCAGGCTTC AGGGCTGGGA CTCAGTCCTG AGGCACTGGG    60
GAGCCATGAG GGGCTGTGGC AGGGAGGGGC AGGGTGTGGA AAGACTCCCC TGGGGCCATG   120
GTGGAGATGT GCTGAGGTCT TCTCCCTGAT CGTCTTCTCC TCCCTGCTGA CCGACGGCTA   180
CCAGAACKAG ATG GAG TCT CCG CAG CTC CAC TGC ATT CTC AAC AGC AAC       229
      Met Glu Ser Pro Gln Leu His Cys Ile Leu Asn Ser Asn
            -35                      -30

AGC GTG GCC TGC AGC TTT GCC GTG GGA GCC GGC TTC CTG GCC TTC CTC       277
Ser Val Ala Cys Ser Phe Ala Val Gly Ala Gly Phe Leu Ala Phe Leu
-25                -20                -15                -10

AGC TGC CTG GCC TTC CTC GTC CTG GAC ACA CAG GAG ACC CGC ATT GCC       325
Ser Cys Leu Ala Phe Leu Val Leu Asp Thr Gln Glu Thr Arg Ile Ala
            -5                        1                        5

GGC ACC CGC TTC AAG ACA GCC TTC CAG CTC CTG GAC HKC ATC CTG GCT       373
Gly Thr Arg Phe Lys Thr Ala Phe Gln Leu Leu Asp Xaa Ile Leu Ala
            10                        15                        20

GTT CTC TGG                                                         382
Val Leu Trp
      25

```

(2) INFORMATION FOR SEQ ID NO: 102:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 279 base pairs
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: DOUBLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo Sapiens
- (F) TISSUE TYPE: Muscle

(ix) FEATURE:

- (A) NAME/KEY: sig_peptide
- (B) LOCATION: 190..273
- (C) IDENTIFICATION METHOD: Von Heijne matrix
- (D) OTHER INFORMATION: score 5.7
seq DHLFLLFPRSCSS/LV

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 102:

```

CTCTTGTTAA CCTGTCTTTT GCTATAGGAG TGTCAGACCC TTATGAGGGG AGAGGAGAGA    60
TATCAIACTT TTTCTACCTC TACACTTTTA ATATCATTA TTTTCTAACA ATGCCCAAAT   120

```

CTTCAGTACA CCTCTCTCTC CTGAACCCTA TACTTGTACA GCAACTTTCT ATGTGACATT 180
 TCTTCTTAA ATG TCT AAT AAG TAT ATC AAA CCT AGC ATG TCC CCA GGA AAC 231
 Met Ser Asn Lys Tyr Ile Lys Pro Ser Met Ser Pro Gly Asn
 -25 -20 -15
 ACT GAT CAT CTT TTC CTA CTC TTC CCC CGA AGT TGT TCC TCC CTC GTC 279
 Thr Asp His Leu Phe Leu Leu Phe Pro Arg Ser Cys Ser Ser Leu Val
 -10 -5 1

(2) INFORMATION FOR SEQ ID NO: 103:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 340 base pairs
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: DOUBLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo Sapiens
- (D) DEVELOPMENTAL STAGE: Fetal
- (F) TISSUE TYPE: kidney

(ix) FEATURE:

- (A) NAME/KEY: sig_peptide
- (B) LOCATION: 263..334
- (C) IDENTIFICATION METHOD: Von Heijne matrix
- (D) OTHER INFORMATION: score 5.6
seq FFFFLFLLPPXPP/TG

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 103:

ATATGTGTAA TGTCTTTATT CCTTAGACTA TGGTCTCCGT GGAAGATTAC TGATACTCCC 60
 ACTAGTATTA ATAACAATGT TAGGTAACAT TACTGAATGT TTACTGAGTG CCAGGTAATG 120
 TTCTAATTGC TTTACATGTA TTAGGCTATG TATTCCTCAC ATGAACCATA TGAAAGAGAT 180
 ACTCTTATTG TTGTCATTTT AGAAGTGAAG AACTGAGGC ACAGAAACT TAAGTAATTA 240
 GTCCAATTCA TACAGGTAGT AT ATG GTA GAA CTG AAG CAG TTG GGC CCC AGG 292
 Met Val Glu Leu Lys Gln Leu Gly Pro Arg
 -20 -15
 TCT TTT TTT TTC TTT CTT TTT CTT CTG CCG CCG RCT CCT CCA ACC GGG 340
 Ser Phe Phe Phe Phe Leu Phe Leu Leu Pro Pro Xaa Pro Pro Thr Gly
 -10 -5 1

(2) INFORMATION FOR SEQ ID NO: 104:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 151 base pairs
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: DOUBLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo Sapiens

(F) TISSUE TYPE: Heart

(ix) FEATURE:

(A) NAME/KEY: sig_peptide

(B) LOCATION: 17..94

(C) IDENTIFICATION METHOD: Von Heijne matrix

(D) OTHER INFORMATION: score 5.5
seq LILPALFFFPLHC/TF

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 104:

```

AAACACCTTC TCAGTG ATG CCT TAC GTC ACC ATC CCA TAT ATA ATA GTG TAC      52
      Met Pro Tyr Val Thr Ile Pro Tyr Ile Ile Val Tyr
            -25                      -20          -15

TCA CTC ATT CTA CCT GCC CTC TTT TTT TTC CCT CTC CAC TGT ACT TTT      100
Ser Leu Ile Leu Pro Ala Leu Phe Phe Phe Pro Leu His Cys Thr Phe
            -10                      -5              1

CAC GGT CTA ACA TAC TAT ATA TCA TGT GTT TGT TCA TTA TCT CTA CCC      148
His Gly Leu Thr Tyr Tyr Ile Ser Cys Val Cys Ser Leu Ser Leu Pro
            5                      10              15

ACG
Thr
                                                    151

```

(2) INFORMATION FOR SEQ ID NO: 105:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 327 base pairs

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: DOUBLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo Sapiens

(D) DEVELOPMENTAL STAGE: Fetal

(F) TISSUE TYPE: kidney

(ix) FEATURE:

(A) NAME/KEY: sig_peptide

(B) LOCATION: 247..321

(C) IDENTIFICATION METHOD: Von Heijne matrix

(D) OTHER INFORMATION: score 5.5
seq LLLCMDLPHSVLS/NW

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 105:

```

AATTATTTTA TAAACTTCT GGCTGGATTT AAATACTAGG CAGTATTCCA AGGGATGATA      60

```

```

AAATGTTTTT ACAAACTTAA TTAGACCCAT TTTTGTAATT AACTTTTATT ATACATGTGC 120
TATGAGGATT AACTTTTGCC TCATAAAAGT ATTCTGACAG GTGCTTTGCA CAGAGTAAGT 180
CCGCCAAAAGT GGACGTTCTC ATATGTAATT CTGAGCTTAC TCATACTGGC CAGGAAGGAC 240
GTGCAC ATG CCA CCT TTG GCA GCT GTG ATG GGG AGC CTG CCT CTG CTC 288
      Met Pro Pro Leu Ala Ala Val Met Gly Ser Leu Pro Leu Leu
      -25                -20                -15

TTG TGC ATG GAC CTT CCA CAT TCT GTC CTG TCC AAC TGG 327
Leu Cys Met Asp Leu Pro His Ser Val Leu Ser Asn Trp
      -10                -5                1

```

(2) INFORMATION FOR SEQ ID NO: 106:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 254 base pairs
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: DOUBLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo Sapiens
- (D) DEVELOPMENTAL STAGE: Fetal
- (F) TISSUE TYPE: kidney

(ix) FEATURE:

- (A) NAME/KEY: sig_peptide
- (B) LOCATION: 186..248
- (C) IDENTIFICATION METHOD: Von Heijne matrix
- (D) OTHER INFORMATION: score 5.5
seq EFLFLGFPSNSWP/HR

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 106:

```

ACAGCTAGAA TATGTTGGAT TCAGGAGCTT GTCCATTATT TGTAGGTAAA AAAAGCTGCA 60
CGTAGATTTG ACTTCAACTC CGTAAAAAAG ACAGCTGTAT TTTCCGTCCA ACTGGAATTG 120
TTGAATCACA CTGCATAGCT GCCCAAAGA GAGTGTGTTGG TCTTGAAGTT TCTATACTTT 180
TATAA ATG TTA CAA ATT CCC GAA AGA AGG GAA TTT CTT TTT CTG GGG TTT 230
      Met Leu Gln Ile Pro Glu Arg Arg Glu Phe Leu Phe Leu Gly Phe
      -20                -15                -10

CCT TCA AAC TCT TGG CCC CAC AGG 254
Pro Ser Asn Ser Trp Pro His Arg
      -5                1

```

(2) INFORMATION FOR SEQ ID NO: 107:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 165 base pairs
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: DOUBLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo Sapiens
- (F) TISSUE TYPE: Muscle

(ix) FEATURE:

- (A) NAME/KEY: sig_peptide
- (B) LOCATION: 49..102
- (C) IDENTIFICATION METHOD: Von Heijne matrix
- (D) OTHER INFORMATION: score 5.5
seq FLITLFCCCVVVG/FF

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 107:

```

ACATGTATCT GTTGGCTATT TGTATATCAT CTTTGCATCT TTGGATAA ATG TTC TTT      57
                                   Met Phe Phe

GTC CAT TTT TTA ATC ACT TTA TTT TGT TGT TGT GTT GTA GTG GGG TTT      105
Val His Phe Leu Ile Thr Leu Phe Cys Cys Cys Val Val Val Gly Phe
-15                -10                -5                1

TTT GGC CAT GAT CAT TCA TTT ATC TCA CAG TTC ATT CTT GTT ACT TGG      153
Phe Gly His Asp His Ser Phe Ile Ser Gln Phe Ile Leu Val Thr Trp
      5                10                15

GCC AGG GCA GGG                                165
Ala Arg Ala Gly
      20

```

(2) INFORMATION FOR SEQ ID NO: 108:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 163 base pairs
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: DOUBLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo Sapiens
- (F) TISSUE TYPE: Heart

(ix) FEATURE:

- (A) NAME/KEY: sig_peptide
- (B) LOCATION: 83..157
- (C) IDENTIFICATION METHOD: Von Heijne matrix
- (D) OTHER INFORMATION: score 5.5
seq CLLHLRCLQLYWA/AR

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 108:

TCC TCC TGC TGT CTT TGC TTG ACC TCC TGC
Ser Ser Cys Cys Leu Cys Leu Thr Ser Cys
-5 1

(2) INFORMATION FOR SEQ ID NO: 110:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 213 base pairs
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: DOUBLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo Sapiens
- (D) DEVELOPMENTAL STAGE: Fetal
- (F) TISSUE TYPE: kidney

(ix) FEATURE:

- (A) NAME/KEY: sig_peptide
- (B) LOCATION: 115..174
- (C) IDENTIFICATION METHOD: Von Heijne matrix
- (D) OTHER INFORMATION: score 5.4
seq VSVSLCVCDCVRG/ST

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 110:

```

ATAAAATTTA CAGAAAAGTT GCAAAGAAGA TAGAATTTCT GCTTAGCTTT TGCCCCAATT      60
TCCCACTTGC CACCCTTCCC TCTTTGTGTT TGTATCTTTT TTTTCTGAG CCAC ATG      117
                                     Met
                                     -20

AAA GTA AAG CCG CCT TTT GTG TCT GTG TCA CTC TGT GTG TGT GAC TGT      165
Lys Val Lys Pro Pro Phe Val Ser Val Ser Leu Cys Val Cys Asp Cys
               -15                      -10                      -5

GTA AGG GGT AGC ACA CTT ACA TGG AAC AGG TTA CTG CGT GTG GGA GGG      213
Val Arg Gly Ser Thr Leu Thr Trp Asn Arg Leu Leu Arg Val Gly Gly
               1                      5                      10

```

(2) INFORMATION FOR SEQ ID NO: 111:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 367 base pairs
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: DOUBLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo Sapiens
- (D) DEVELOPMENTAL STAGE: Fetal
- (F) TISSUE TYPE: kidney

(ix) FEATURE:

- (A) NAME/KEY: sig_peptide
- (B) LOCATION: 68..184

(C) IDENTIFICATION METHOD: Von Heijne matrix
 (D) OTHER INFORMATION: score 5.4
 . seq ILLTSCFYTLVSS/TF

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 111:

```

ATGGCTAACA TATTCTTTTT TTTTCTCTG TAGTAGTTTT TTGAAAGAAG AAATAGGCTA    60
TTCTAGC ATG ATC TCA TCC TGT GGA GTT AAA TAC TTG TTT TCA CAT GCC    109
      Met Ile Ser Ser Cys Gly Val Lys Tyr Leu Phe Ser His Ala
      -35                                -30
TCC TTA TTT TTT ATG GTA GGG AGT ACA GGA AGT TTA ATA CTC TTA ACT    157
Ser Leu Phe Phe Met Val Gly Ser Thr Gly Ser Leu Ile Leu Leu Thr
-25                                -20                                -15
TCT TGT TTC TAT ACC CTT GTT TCA TCA ACC TTT CTT CAA AAA CTC TCT    205
Ser Cys Phe Tyr Thr Leu Val Ser Ser Thr Phe Leu Gln Lys Leu Ser
      -5                                1                                5
TCT TTG CTC TTG ATA TTA TTT ACC GAA ACA AGT GTY CTT ATG TTA AAA    253
Ser Leu Leu Leu Ile Leu Phe Thr Glu Thr Ser Val Leu Met Leu Lys
      10                                15                                20
ACA TTT GTA GCT AAT TCT TGC TGT WAA TTG TGG TCT CAC AAT TGT ATT    301
Thr Phe Val Ala Asn Ser Cys Cys Xaa Leu Trp Ser His Asn Cys Ile
      25                                30                                35
AAT TTC TTC AAA AAG GTC CKG CCT TCT TAT TGC KGC AGC AGT CTA CTC    349
Asn Phe Phe Lys Lys Val Xaa Pro Ser Tyr Cys Xaa Ser Ser Leu Leu
      40                                45                                50                                55
TTC CTG GCC GTA CCT AGG
Phe Leu Ala Val Pro Arg    367
      60
  
```

(2) INFORMATION FOR SEQ ID NO: 112:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 248 base pairs
 (B) TYPE: NUCLEIC ACID
 (C) STRANDEDNESS: DOUBLE
 (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:
 (A) ORGANISM: Homo Sapiens
 (F) TISSUE TYPE: Muscle

(ix) FEATURE:
 (A) NAME/KEY: sig_peptide
 (B) LOCATION: 174..233
 (C) IDENTIFICATION METHOD: Von Heijne matrix
 (D) OTHER INFORMATION: score 5.4
 seq SFLCNFLVSLSL/FL

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 112:

```

AGAAGGGGGT GAAAGGAGTA ACTGCTATAT TTAGAAGGAG GTTAAGGATA GCAATTGATT    60
TTAAGGGTGG GGCTAGGGAA CTTGTCTTTA AAATCCTGCA TTTGCACAGC AAGCACAGTT    120
CGTATTGAGA TTTTGCTATT TGGAAGTGTG AGGGAGGTAT AGGATGCTGC CTA ATG      176
                                   Met
                                   -20

GGA GGT GGG ATH GCA GAG AGT TTT CTA TGT AAT TTC TTG GTA TCA CTT      224
Gly Gly Gly Ile Ala Glu Ser Phe Leu Cys Asn Phe Leu Val Ser Leu
               -15                      -10                      -5

TCC CTC TCT TTC CTC CAT GGC CGG                                      248
Ser Leu Ser Phe Leu His Gly Arg
               1                      5

```

(2) INFORMATION FOR SEQ ID NO: 113:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 408 base pairs
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: DOUBLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo Sapiens
- (D) DEVELOPMENTAL STAGE: Fetal
- (F) TISSUE TYPE: kidney

(ix) FEATURE:

- (A) NAME/KEY: sig_peptide
- (B) LOCATION: 265..363
- (C) IDENTIFICATION METHOD: Von Heijne matrix
- (D) OTHER INFORMATION: score 5.4
seq LAYFLCCQGVIFG/SL

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 113:

```

CTATTTCTCA TTGTCTGTCT GGTTTTCCAT CCCCTCACA TGTGGTGACC AGCACCTGGC    60
CCGCCACGGC AGCCAGGAGG CATTTGTAA GCGAATAATC GAGACAGGGA AGAGGAGTGG    120
AGTTGGCTGC TCCAGACTCT GCTTAGTTTT CCTTTCTCAA AGTTCTCCCT CCTGTGTCCT    180
AGCCGGGGAA TTAGCTAAAA TGGAATTTTC TTTGGTGATC AGGTATCCTT CTGATGAAGA    240
GAAGAAAGGC CTAACTCCC AGGC ATG GAT GCA TTA GAA AGA GGT AGT CTT      291
                                   Met Asp Ala Leu Glu Arg Gly Ser Leu
                                   -30                      -25

AGA AAT GAG CAG GCG TTG GTT ATT TAT GCA GGA CTG GCA TAC TTT CTG      339
Arg Asn Glu Gln Ala Leu Val Ile Tyr Ala Gly Leu Ala Tyr Phe Leu
               -20                      -15                      -10

```

TGC TGC CAA GGG GTG ATT TTT GGA AGT CTC CCC TCT AAT GCT GGT GCT	387
Cys Cys Gln Gly Val Ile Phe Gly Ser Leu Pro Ser Asn Ala Gly Ala	
-5 1 5	
GGG CCT TTG GGA TGG TCT AGC	
Gly Pro Leu Gly Trp Ser Ser	408
10 15	

(2) INFORMATION FOR SEQ ID NO: 114:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 209 base pairs
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: DOUBLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo Sapiens
- (F) TISSUE TYPE: Muscle

(ix) FEATURE:

- (A) NAME/KEY: sig_peptide
- (B) LOCATION: 78..194
- (C) IDENTIFICATION METHOD: Von Heijne matrix
- (D) OTHER INFORMATION: score 5.3
seq SLWFLPLPHVYT/HT

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 114:

TTGCTTGAAC CTAAGTGTCT TGTTTTTGTC TTCCTGTGAG TTCAAGGACA GGAGCAGTGC	60
TTAACACACA GTAGGTA ATG GAA TAT TTG TTC CAG CAG CCT GGA CAC TCA	110
Met Glu Tyr Leu Phe Gln Gln Pro Gly His Ser	
-35 -30	
AGG GGA GAA GCC AGG GCT GCT GCT GCC TCT CTG GAA ACC CTG TCT TCC	158
Arg Gly Glu Ala Arg Ala Ala Ala Ala Ser Leu Glu Thr Leu Ser Ser	
-25 -20 -15	
CTT TGG TTT CTG CCT CTC CCA ACC CAC GTG TAC ACA CAT ACA CAT GCC	206
Leu Trp Phe Leu Pro Leu Pro Thr His Val Tyr Thr His Thr His Ala	
-10 -5 1	
AAC	
Asn	209
5	

(2) INFORMATION FOR SEQ ID NO: 115:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 387 base pairs
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: DOUBLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo Sapiens
- (F) TISSUE TYPE: Dystrophic muscle

(ix) FEATURE:

- (A) NAME/KEY: sig_peptide
- (B) LOCATION: 283..327
- (C) IDENTIFICATION METHOD: Von Heijne matrix
- (D) OTHER INFORMATION: score 5.3
seq SSMLITILSFIFA/LG

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 115:

```

ACCACAGTCA CTGTCACATT ATTCTGTTTT GTATTTTATT TACAGCTCTT ATAATTATCC   60
GAACTTACAA ATTTATTTTC TTGTGTTTTT TCCGCCTGCT CCTCCACTTC ATTCTGTAAT  120
ACTATAGTTC ACTATAATAC TTCTAGTTCC TAGGACTGGA ATTATGTGTC TGGCACATAG  180
TAGACAGTAG ATGTTCAATG AATGAATGAA TGATTCAAAT GAGATTTAAA TAGCAACAGT  240
CCTGACAGAA TGGTAAATTT CCACACTTAA GATGGTCTGT TA ATG GTA TCA TCA      294
                               Met Val Ser Ser
                               -15

ATG TTG ATA ACT ATT CTA TCG TTT ATT TTT GCC TTA GGG TAC CAC ACA      342
Met Leu Ile Thr Ile Leu Ser Phe Ile Phe Ala Leu Gly Tyr His Thr
-10                               -5                               1                               5

GCT TCT TAT CCA GTC TCC CTT CAT CCA CTC TCC TTT TTC CTA CAC          387
Ala Ser Tyr Pro Val Ser Leu His Pro Leu Ser Phe Phe Leu His
          10                               15                               20

```

(2) INFORMATION FOR SEQ ID NO: 116:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 405 base pairs
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: DOUBLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo Sapiens
- (D) DEVELOPMENTAL STAGE: Fetal
- (F) TISSUE TYPE: kidney

(ix) FEATURE:

- (A) NAME/KEY: sig_peptide
- (B) LOCATION: 316..369
- (C) IDENTIFICATION METHOD: Von Heijne matrix
- (D) OTHER INFORMATION: score 5.3
seq MNLVSALASSAXG/QR

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 116:

```

ACAGTACTTG GAGGTATTCT AAAGGCAGAC ATACTTTATC TGAGCAGGTG CTTTGGCGT    60
GGTCCTGCCA AGAAAGAAAC AATGGCTTAG ATGACGTCTA TTCTAAGGCC TCAAGGCTTG    120
CACCCCTGCC ATGCTAAATA CAGATGCGCT CCTCCACCAA GAGAATCCCC TCTGCCCTCT    180
GCCATCTCAG CCCCAGGCCA GCTCAGCTGC CCATGACCTG TGTGCAAAGC AGGGGGCGGG    240
ACAAACAGCT ATCGCCTTTG GCCTTCCCTT TGCTCCTGAC AGCGGTCTCA AACCTGGAGG    300
AGTCAAAGGT CCAAG ATG CCT TTG TTC ACT ATG AAC CTG GTG TCA GCT CTA    351
          Met Pro Leu Phe Thr Met Asn Leu Val Ser Ala Leu
                    -15                               -10

GCG TCC TCA GCA RCA GGG CAG CGT GGA GCA GGG CCA GCC CTC TGG CAC    399
Ala Ser Ser Ala Xaa Gly Gln Arg Gly Ala Gly Pro Ala Leu Trp His
   -5              1              5              10

TTG TGT
Leu Cys                                         405

```

(2) INFORMATION FOR SEQ ID NO: 117:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 232 base pairs
 (B) TYPE: NUCLEIC ACID
 (C) STRANDEDNESS: DOUBLE
 (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:
 (A) ORGANISM: Homo Sapiens
 (D) DEVELOPMENTAL STAGE: Fetal
 (F) TISSUE TYPE: kidney

(ix) FEATURE:
 (A) NAME/KEY: sig_peptide
 (B) LOCATION: 110..226
 (C) IDENTIFICATION METHOD: Von Heijne matrix
 (D) OTHER INFORMATION: score 5.2
 seq LILLHCSIRVFF/FF

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 117:

```

CTTGCTTGTA AACATAAGCA TGTATTATTA CCTAGGCTTT GAATTTCAAA ATACGGTGTA    60
AACTACTCAT GGTAATATAG ATCTTGTTAG ACAAACGTTT ATGTAAAAA ATG ATC TGC    118
                               Met Ile Cys

AAG CAT TAC TGT ATA AAG AAA AAT AAC CTG GAT TAC TTG AAT AGA ATG    166
Lys His Tyr Cys Ile Lys Lys Asn Asn Leu Asp Tyr Leu Asn Arg Met
-35              -30              -25

GTT TAC AST GCT CAG TTA AAG TTG ATA CTT CTT CTA CAT TGC AST ATT    214

```

Val Tyr Ser Ala Gln Leu Lys Leu Ile Leu Leu Leu His Cys Ser Ile
 -20 -15 -10 -5

AGG GTT TTT TTT TTT TTT
 Arg Val Phe Phe Phe Phe
 1

232

(2) INFORMATION FOR SEQ ID NO: 118:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 429 base pairs
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: DOUBLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo Sapiens
- (D) DEVELOPMENTAL STAGE: Fetal
- (F) TISSUE TYPE: kidney

(ix) FEATURE:

- (A) NAME/KEY: sig_peptide
- (B) LOCATION: 232..390
- (C) IDENTIFICATION METHOD: Von Heijne matrix
- (D) OTHER INFORMATION: score 5.2
seq SLLLLQLIHEDKA/IQ

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 118:

AATTTGAGAA GTGCCCTCCT ATACTTAGAG AAAAGGAATA TCCATATCTC TGAAGACACA 60
 GGGACACAGA GAGAATCTGA ACACACAGCC TTGGTAGGAT TCCTTCCGTT TATCATCATT 120
 AGATCATAAC CCCYTTTGTC MAGTCCTATT TCTCCARGAC TGCCTCCTTC TTCATTAAAC 180
 CTTGCATAAA AACTCACAAA TTAAACCATT TATTTGGATT CTTATTTCTT T ATG AAA 237
 Met Lys
 ATT CCT GTG TGG CAT AAA ACG TGC TTT TTA AAA TCT GAA AGT TTT TCT 285
 Ile Pro Val Trp His Lys Thr Cys Phe Leu Lys Ser Glu Ser Phe Ser
 -50 -45 -40
 CCT GAT AAT TTA TCT GTT AGT TTG CCT TGT AGA CCT AGC CAG GTA CCC 333
 Pro Asp Asn Leu Ser Val Ser Leu Pro Cys Arg Pro Ser Gln Val Pro
 -35 -30 -25 -20
 TCA CAG GGG CAA GGA AAA TCT TTT CTC CTC CTA CAA CTT ATA CAT GAG 381
 Ser Gln Gly Gln Gly Lys Ser Phe Leu Leu Leu Gln Leu Ile His Glu
 -15 -10 -5
 GAT AAA GCC ATC CAG AAT GAA GCT ATT TTC CAG CCT TCT CTG CAG CTG 429
 Asp Lys Ala Ile Gln Asn Glu Ala Ile Phe Gln Pro Ser Leu Gln Leu
 1 5 10